UNIVERSITY OF EDINBURGH

A STUDY OF ENZOOTIC STAPHYLOCOCCAL INFECTION IN LAMBS, WITH SPECIAL REFERENCE TO PYAEMIA ASSOCIATED WITH TICK-BITE.

Ph.D. THESIS

presented by

ARCHIBALD McDIARMID, M.R.C.V.S.

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INTRODUCTION.

Diseases of a suppurative or pyaemic nature are of common occurrence in sheep throughout Britain. Thus, Jowett (1930) describes a pyobaccillosis caused by infection with Bacterium purifaciens. This condition is mainly encountered in mature sheep and has a pyaemic distribution with superficial suppurative lesions in the submaxillary and parotid regions and also deeply placed abscesses in viscera such as the lungs and kidneys. Cornell and Glover (1925) describe two types of joint-ill in lambs, one caused by a streptococcal infection, the other by Erysipelothrix rhusiopathiae. The streptococcal type mainly affects lambs about seven to ten days old, whereas infection with E. rhusiopathiae usually occurs in older lambs. Menzies (1938) and Blakemore (1939) record the occurrence of streptococcal joint-ill in lambs in the south western and eastern counties of England respectively. True streptococcal joint-ill may affect the viscera in addition to the joints after the preliminary multiplication of the organisms in the systemic circulation.

In the course of routine post mortem examinations at this Institute, Corynebacterium pyogenes has been isolated by the author from suppurative foci in the thoracic cavity of rams which have been housed during the winter months. It has also been observed that a necrobacillosis caused by Fusiformis necrophorus occurs in young lambs. This infection, which apparently

arises /

arises as a result of umbilical infection, is essentially pyaemic in its distribution, the organisms localising in the liver and lungs and producing large necrotic lesions at these predilection sites.

In addition to the infections which have been outlined above and which affect sheep on arable land, there occurs on many of the tick-infested hill farms of Britain a serious enzootic disease of lambs which is responsible for much death and debility in these animals during the first few weeks of life. This infection is apparently associated with tick-bite and may develop concurrently with other diseases of hill lambs such as: lamb dysentery, pulpy kidney disease, louping-ill and tick-borne fever. When this occurs it is difficult at autopsy to decide which infective agent may have been responsible for the fatal outcome.

This staphylococcal infection is variously known as 'cripples', joint-ill, pyaemia, pyaemic spinal meningitis and tick pyaemia. During investigations in Northumberland for the purpose of obtaining information regarding the cause of louping-ill McFadyean (1894) encountered this crippling condition which he named pyaemic spinal meningitis. It is interesting to note that these lambs brought to McFadyean for autopsy, were considered to be typical 'louping-ill' cases by the shepherds in that area, and McFadyean remarks:- "At the present day, both in England and in Scotland, shepherds appear, like Fair, to /

to regard louping-ill as a disease that assumes many different forms, for during the months of April and May they call every case of illness in the flock louping-ill, when the subject is found paralysed or unable to stand." McFadyean's reference to Fair (1839) indicates the latter's observations which McFadyean quoted in his own article as follows:- "Some few instances, however, have occurred among my flock when they have most unexpectedly recovered, so as to again follow their neighbours and get entirely well; and in other cases they have for a length of time dragged a seemingly powerless hind leg behind them, and the left leg oftener than the right one. When this, however, takes place, the limb still remains cold and dead, for a time, in despite of the use of friction or stimulants. If it is a fore leg it is not uncommon, after the sheep gets on its feet again, for a tumour of the size of a pigeon's or even a hen's egg, filled with pus or ichor to appear. On being punctured it presently subsides and is lost. These abscesses usually appear in the neighbourhood of the joints, but sometimes about the arms, the brisket, or any neighbouring part of the body." The above statement suggests the possibility that Fair was dealing with this crippling condition because the joints and sternal cartilages are common predilection sites for the formation of the suppurative lesions. The following is another quotation from McFadyean's paper:- "Without /

"Without attempting to decide which of them has the best claim to the name, I feel warranted in saying that the following are the principal morbid conditions at the present time grouped under the heading of louping-ill:-

i) Pyaemic spinal meningitis, caused by pyogenic bacteria,

ii) Gastritis and Enteritis from indigestible substances (wool, sand, dried grass) in the stomach or intestines,

iii) Disorders of brain functions, paralysis and general weakness, with, in some cases, excess of cerebro-spinal fluid in the cranial cavity, but without gross lesions in any of the organs of the body.

The first two of these conditions cover most of the cases of louping-ill in lambs, and the third includes the bulk of the cases in adult sheep. The cause or causes of the last of these groups of cases have not yet been satisfactorily determined, but all the experiments hitherto made indicate that these cases are not bacterial or transmissible by inoculation."

Therefore there can be little doubt that this crippling condition and true louping-ill are closely connected from the clinical point of view. In fact, clinically, without post mortem evidence it is extremely difficult in certain cases to distinguish between these two conditions unless superficial or palpable abscess formation has taken place.

McFadyean (1894) described the isolation of a slightly chromogenic Gram-positive micrococcus from some of his cases and cultures of this pyogenic organism were /

were employed by him for attempted transmission of the disease in sheep; local abscesses were readily produced but there was no evidence of the occurrence of pyaemic lesions in any of the experimental animals. McFadyean, nevertheless, when discussing his findings, makes the following important statement:- "On the other hand, it is very probable that by injecting cultures of this organism into the veins of young lambs, so as to insure the transport of the germs by the blood stream and thus bring about a chance of their becoming arrested in one of the bones of the spinal column, cases identical as regards the symptoms with II and V would be produced." II and V refer to two typical cases of the disease encountered by McFadyean.

Stewart and Ponsford (1937) confirmed McFadyean's observations regarding the morphology of the organism. They referred to pyaemic infection of lambs as 'tick pyaemia' and indicated that the disease was very prevalent on tick-infested pastures. Taylor et al. (1941) found that the organism involved resembled Staphylococcus aureus, reviewed the incidence of 'pyaemia' and showed it to be the cause of considerable loss in the lamb population of many tick-infested farms in Scotland. They also inferred that its occurrence was in some way associated with tick-bite because it only occurred in lambs in tick-infested areas and because /

because staphylococci could be isolated both from superficial abscesses at the site of tick-bite and also from deeply placed lesions in the same animal. Subcutaneous inoculation of normal lambs and lambs suffering from louping-ill and/or tick-borne fever with cultures of the organism failed to produce pyaemia. Apart from these papers, little evidence regarding the disease can be found in the literature.

The synonyms by which the infection is known are somewhat confusing. For example 'pyaemic spinal meningitis' refers only to that phase of the infection in which the coverings and surrounding structures of the spinal cord are involved in the suppurative process. Thus this term can hardly be suitably employed to describe the disease in general. 'Cripples' and 'joint-ill' are somewhat descriptive terms but are apt to be misunderstood, since numerous infections which have already been mentioned may give rise to crippling joint conditions in lambs. 'Pyaemia' is not a satisfactory term, since it denotes the final phase of the disease and does not indicate the nature of the infective agent, whilst 'tick pyaemia' is not appropriate for the purpose of describing a disease of lambs.

More recently during the continued investigation of this infection we have encountered a more acute form of the disease which can only be diagnosed by bacteriological methods owing to the absence of characteristic /

characteristic pyaemic lesions. Because of this more acute form of the condition and because of the widespread occurrence and high incidence of the disease on tick-infested farms, the term 'enzootic staphylococcal infection of lambs associated with tick-bite' is suggested as an appropriate designation for this disease.

DISTRIBUTION OF THE DISEASE.

Enzootic staphylococcal infection only occurs in lambs on tick-infested pastures and has been recognised in Scotland, England and more recently Ireland (Foggie 1943). Thus the disease is comparatively widespread throughout the British Isles where ever the common sheep tick Ixodes ricinus L. is found.

BREED SUSCEPTIBILITY.

Lambs of all breeds common to hill grazings are liable to contract infection provided they are exposed to tick-infestation. During the present study the disease has been encountered in Blackface, Cheviot, Half-bred and Greyface lambs.

AGE INCIDENCE.

The pyaemic lesions are chiefly seen in lambs between a fortnight and one month of age, but the more acute septicaemic type of the disease affects lambs soon after birth. In fact, from the appearance of the visible lesions in the more chronic cases and taking the ages of the affected animals into consideration, it is indeed probable /

probable that most lambs contract infection soon after birth, possibly within the first week of life. Since lambs even a day old may harbour large numbers of ticks the opportunity for early infection can be readily appreciated.

There is no evidence of the occurrence of the disease in hoggs or older sheep. This suggests that immunity develops with advancing age and is probably the result of active association with the infective agent in early life.

THE INCIDENCE OF THE INFECTION ON TICK-INFESTED PASTURES.

It is worthy of note that the diagnosis of enzootic staphylococcal infection has so far depended upon the presence of macroscopic suppurative lesions in the lamb. Thus it is highly probable that if a bacteriological examination had been carried out in each instance, more acute staphylococcal infection would have been recognised. Despite the fact that many cases of the disease may not have been recorded, the incidence of the infection is nevertheless comparatively high as indicated by previous authors.

McFadyean (1894) mentioned that half of the so-called louping-ill cases brought to him were actually cases of pyaemic spinal meningitis. Stewart and Ponsford (1937) record that:- "of 209 lambs examined after death on tick-infested pasture, pyaemic infections were noted in 100 cases and in all probability were the cause of death in 80 cases."

Taylor et al. (1941) during investigations extending over a period of two years found the incidence of the disease to be 29 per cent. in lambs brought for post mortem examination.

Apart from the lambs which die from this infection, the disease is responsible for much debility, paralysis and chronic lameness.

It is difficult to assess the precise number of deaths which can be attributed to staphylococcal infection per se. The reason for this difficulty is the occurrence of different diseases which may develop concurrently with staphylococcal infection. There can be little doubt, however, that this enzootic disease is one of the major disease problems encountered in young lambs.

SEASONAL INCIDENCE.

Since the disease is associated with tick-bite, it is seasonal in incidence and occurs in spring when ticks are most active. The majority of cases occur in April, May and the beginning of June and it is extremely rare to encounter anything but chronic cases later in the year. When a case is met with after the end of June - it is usually of a retrogressive nature, the fibrosed appearance of the lesions indicating that the animal was infected a considerable time prior to examination.

THE ASSOCIATION OF STAPHYLOCOCCAL INFECTION WITH OTHER DISEASES IN THE SAME ANIMAL.

Stewart and Ponsford (1937) record that Clostridium welchii type D infection may co-exist with pyaemia, and Taylor et al. (1941) mention the close association between louping-ill, tick-borne fever and pyaemia.

The accurate differential diagnosis of the principal infections of lambs on tick-infested pastures is dependant on laboratory findings. Therefore, in addition to recording any macroscopic abnormality, the following methods were adopted by the author during the course of routine post mortem examinations at this Institute.

1. LOUPING-ILL:

Biological Test:- If the lamb were alive and had a febrile reaction, blood was withdrawn from the jugular vein into 3 per cent. sodium citrate solution. This mixture was diluted 1 in 5 with normal saline and injected intracerebrally in mice in 0.05 c.c. quantities. When a dead lamb was examined small portions of cerebrum, cerebellum and medulla were pulped in a sterile mortar and normal saline was added so as to give a 1 in 100 suspension of brain tissue. Mice were inoculated intracerebrally with 0.05 c.c. of this suspension.

If virus was present in detectable amounts in the blood or brain tissue, the mice inoculated with either substance developed typical louping-ill within five to nine days after inoculation.

Histological Examination:- As a routine procedure, apart /

apart from mouse inoculation, sections were prepared from the cerebrum, cerebellum and medulla of any lamb suspected of having been affected with louping-ill. These were stained by haematoxylin and eosin. Lesions of an encephalo-myelitis, including migration of round cells and neuronophagia, were considered to be indicative of the disease.

2. TICK-BORNE FEVER:

Occasionally a lamb which had a febrile reaction was brought to the laboratory and in those circumstances the infective agent could be observed in a blood film obtained from the patient. If, however, the lamb's temperature was normal, a susceptible sheep was inoculated subcutaneously with 20 c.c. of the lamb's blood. In the case of tick-borne fever, the inoculated animal developed a temperature reaction about four to six days after injection. A blood film was prepared from the experimental sheep during this febrile phase and stained by Leishman's stain. This film was examined microscopically for the presence of the infective agent of tick-borne fever in the polymorphonuclear leucocytes.

Apart from animal inoculation, smears prepared from the spleens of dead lambs were occasionally helpful in diagnosing the condition.

3. ENZOOTIC STAPHYLOCOCCAL INFECTION:

Smears and cultures were prepared from the heart blood and liver of the dead lamb or from any organ which showed /

showed evidence of suppuration. The smears were stained by Gram's stain and examined for the presence of staphylococci. The cultures were made upon five per cent. sheep blood agar medium and the plates were incubated at 37°C. for twenty-four hours. If colonies of the organisms were obtained they were tested for coagulase production and haemolytic activity. These tests will subsequently be described indetail in the present paper.

4. PULPY KIDNEY DISEASE:

The contents of the small bowel were filtered through paper pulp until a clear fluid was obtained. This was injected in 0.5 c.c. quantities into mice intravenously.

If the filtrate proved toxic, the toxin present was typed by mixing the filtrate with different types of antitoxins *in vitro* and inoculating mice intravenously from each mixture after the toxin and antitoxin had been in contact for at least thirty minutes. The antitoxins employed were *Cl. welchii* types A, B and D. The lamb was considered to have been affected with pulpy kidney disease if type D toxin was identified in the bowel filtrate.

In addition to the above method of diagnosis, cultural tests were sometimes employed. Tubes of cooked meat (horse flesh) medium were inoculated from the intestinal contents of the lamb. After four days incubation at 37°C. the supernatant fluid was pipetted off from these cultures /

cultures and tested for the presence of Cl. welchii toxins by mouse inoculation.

5. ACUTE LAMB DYSENTERY:

The methods employed for diagnosing this condition were similar to the above. A bowel filtrate was prepared and examined for toxicity. If toxin was detected, toxin-antitoxin mixtures were injected intravenously in mice as previously described. Acute lamb dysentery was diagnosed if type B toxin was detected in the bowel contents of the affected lamb.

Cultural tests similar to those used for diagnosing pulpy kidney disease were also occasionally employed.

During the present investigation of enzootic staphylococcal infection, numerous post mortem examinations have revealed the fact that a variety of different associations between diseases on tick-infested farms is possible. Thus the following cases are typical of a series of tick-infested lambs examined by the author during the spring months of 1941 and 1942.

Case I. A Cheviot lamb of about two weeks of age heavily infested with ticks. The animal was dead when brought to the laboratory.

Post-mortem Findings. The liver was slightly fatty and the left kidney, which was enlarged, contained a quantity of purulent material especially in the vicinity of the pelvis. The right precrural lymphatic gland was enlarged and haemorrhagic.

The /

The bowel contents were examined for toxicity in mice. No toxin was detected. The brain was examined histologically for lesions of louping-ill and a suspension of brain tissue was inoculated intracerebrally in mice. No evidence of the presence of louping-ill was obtained.

Blood agar plates were inoculated from the kidney and also from the heart blood. Both these sites yielded pure cultures of a coagulase-positive haemolytic staphylococcus whose characters were typical of the strains usually encountered in lambs.

Conclusion. Enzootic staphylococcal infection.

Case 2. A Cheviot lamb of about four weeks of age. Numerous ticks adherent to the inner aspect of both fore and hind legs.

The animal was moribund when brought to the laboratory and it was killed by bleeding after 20 c.c. of blood had been withdrawn from the jugular vein. This blood was immediately inoculated subcutaneously into a normal sheep.

Post-mortem Findings. Numerous abscesses present in the liver substance. Small abscesses present in all lobes of the lungs. Fibrinous pericarditis. Abscess formation in left hock joint. From all these lesions coagulase-positive haemolytic staphylococci were obtained in pure culture.

As in Case 1, the bowel contents were examined for toxicity /

toxicity and the brain for the presence of louping-ill. Neither toxin nor louping-ill virus was detected.

The sheep which had been inoculated with the lamb's blood developed a febrile reaction on the 5th day after inoculation, and a blood film taken at the height of the febrile reaction revealed the presence of the infective agent of tick-borne fever.

Conclusion. Enzootic staphylococcal infection and tick-borne fever.

Case 3. A Half-bred lamb aged three weeks. The animal was comatose and heavily infested with ticks. Small local abscesses were present in the vicinity of the tick-bites. The lamb was killed by bleeding and a sample of blood inoculated into a normal sheep. No febrile reaction occurred.

Post-mortem Findings. The liver, kidneys and lungs were all congested. Excess of pericardial fluid was present in addition to endocardial haemorrhages. A bowel filtrate was prepared and injected into mice intravenously. The mice died and subsequent neutralisation tests indicated that Cl. welchii type D toxin was present in the bowel contents. Media inoculated from the heart blood and liver yielded pure cultures of coagulase-positive haemolytic staphylococci.

Conclusion. Enzootic staphylococcal infection and pulpy kidney disease.

Case 4. A Cheviot lamb of about four weeks of age. The animal was dead when brought to the laboratory.

Post-mortem Findings. The liver was slightly fatty, and /

and subcapsular petechiae were found in the kidneys. Both lungs were very congested and contained numerous haemorrhages. Cultures from the liver indicated the presence of coagulase-positive haemolytic staphylococci. No toxin was detected in the bowel contents. Mice inoculated with an emulsion of brain tissue developed typical louping-ill after a period of 7 days.

Conclusion. Enzootic staphylococcal infection and louping-ill.

Case 5. A Blackface lamb about two weeks old heavily infested with ticks. The animal was dead when brought for examination.

Post-mortem Findings. Petechial haemorrhages in kidneys and lungs. A few epicardial petechiae. Congestion of small intestine. Histological examination of the central nervous system and inoculation of mice with brain tissue failed to reveal the presence of louping-ill. Cultures from the liver yielded coagulase-positive haemolytic staphylococci. A bowel filtrate was prepared and when inoculated intravenously into mice proved toxic. Toxin-antitoxin tests indicated that the toxin present was Cl. welchii type B.

Smears from the spleen did not show any evidence of tick-borne fever.

Conclusion. Enzootic staphylococcal infection and lamb dysentery.

From these cases it will be observed that staphylo-

coccal infection can occur by itself or co-exist with at least four other infections. Indeed, it is quite possible to find more than two infections in the one lamb. For example it is feasible that tick-borne fever would have been found in cases 1, 4 and 5 if suitable methods had been employed for detecting it. In cases 1 and 4 no attempt was made to diagnose tick-borne fever and in case 5 little importance can be attached to the fact that the smears made from the spleen tissue were found to be negative. Only by the inoculation of susceptible sheep with blood from the patient could a negative diagnosis of tick-borne fever be given.

These examples illustrate the difficulty which may be experienced in reaching a diagnosis as to the exact cause of death. Thus a staphylococcal infection was probably responsible for the fatal outcome in cases 1 and 2, but cases 3, 4 and 5 were infected with a fatal disease in addition to the staphylococcal infection and death was probably caused by the combined infection. In considering the possible cause of death in a lamb which is affected with staphylococcal infection in addition to some other disease, two factors should be borne in mind. Firstly, the invasive character of the staphylococcus and its power to produce toxins in the acute cases where no abscessation may be visible, and secondly, in the more chronic phase of the disease the impairment of function of a vital organ such as the heart /

heart as a result of the presence of a pyogenic lesion in the myocardium. These factors can no doubt be considered as important contributory causes towards the death of an affected lamb, even if the staphylococcal infection per se may not be responsible for the fatal outcome in every instance.

THE ROLE OF THE TICK AND THE PROBABLE SOURCE OF INFECTION.

As already mentioned the disease only occurs in lambs on tick-infested farms and the causal organism can be isolated from both superficial and deep lesions in an affected lamb. The role of the tick in the production of the disease has so far not been determined, but in the course of the present investigation, eight cultures of haemolytic staphylococci have been isolated from eleven ticks adherent to pyaemic lambs. No staphylococci were obtained from four ticks attached to non-infected animals.

The superficial lesions which appear on the skin or in the subcutaneous tissue of an affected lamb are usually at the site of tick-bite and it has been noted that the umbilicus, a favourite site for the entrance of pathogenic organisms, has rarely been found to be inflamed or suppurative in any of the numerous lambs examined at this Institute over a period of two years. Since one of the principal sites of tick-bite is in the inner aspect of the fore and hind legs, where the skin is thin and not covered by dense wool, it appeared

possible /

possible that the organisms might gain direct access to the systemic circulation by means of the numerous small superficial veins in this area and finally become embedded in the capillary walls in the various organs. This would be aided to a considerable extent by the anti-coagulating secretions from the tick. Moreover since the susceptibility of young lambs is probably high as indicated by pathogenicity experiments described later in this paper, a very minute quantity of organisms might be sufficient to produce the onset of the disease. The other alternative is that infective emboli, from the original abscess at the site of the tick-bite, are liberated as a result of the breakdown of the infective focus, but in connection with this theory it must be borne in mind that the local reaction, due to the resistance of the body, for example the infiltration of phagocytic cells and the formation of fibrous tissue, in addition to the part played by the organisms such as the production of coagulase and leucocidin, must in itself be of such a nature as to provide a comparatively substantial barrier to the progress of infection from the original lesion.

The source of the organisms may even be the skin of the lamb itself and a study of the types of staphylococci present on the integument of the lamb at various intervals after birth in addition to a survey of the organisms in the vagina of the ewe, might provide some useful information on this subject. The incidence of staphylococcal mastitis in hill ewes is not sufficient

to /

to warrant that source being incriminated as the origin of the infection. Much has yet to be done in determining the precise source of the organisms and in assessing the true significance of the tick in the causation of the disease. The fact that the lamb at various stages in its early life is subjected to conditions which are suitable for the infection of the tissues such as the torn umbilicus, the docked tail and castration wounds, rather suggests that the infection does not arise merely from superficial injury since the foregoing injuries would theoretically appear to be far more conducive to infection than the superficial punctures produced by the ticks. Moreover, on farms free from tick-infestation, lambs, which are subjected to the same types of mechanical injury, do not contract enzootic staphylococcal infection although in some instances the operation wounds become suppurative. In view of this it is possible that the tick may act as a vector of the staphylococcus rather than as a mechanical inoculator of organisms which may be present on the skin of the lamb.

CLINICAL AND PATHOLOGICAL FINDINGS ASSOCIATED WITH THE NATURALLY OCCURRING DISEASE.

Enzootic staphylococcal infection can be recognised in two main forms.

i) The Acute or Septicaemic type. Death is sudden, the lamb rarely being found alive and the carcase is invariably /

invariably tick-infested. Autopsy reveals the presence of numerous petechiae in the majority of the viscera and haemorrhages in the epi- and endocardium. The lungs frequently contain large numbers of small congested areas and evidence of inflammation may be found in the liver, spleen and kidneys. Excess pericardial, pleural or peritoneal fluid is encountered in some cases. The whole pathological picture rather suggests a toxaemia, and the diagnosis of staphylococcal infection can only be established by the isolation of the infective agent, together with the careful elimination of acute lamb dysentery, pulpy kidney disease, louping-ill and tick-borne fever.

ii) The Chronic or Pyaemic Type. If a lamb which becomes infected with the staphylococcus survives infection for 48 hours it is likely that a pyaemic case will result. At this stage the infection is usually noted by the shepherd who observes that the lamb is stiff and unwilling to follow the ewe. The animal lies down frequently and appears to be in some considerable discomfort. On examination it is frequently found that one or more of the limb joints are enlarged due to a fluctuating swelling which is hot and painful to the touch. The lamb may salivate profusely, and although not willing to move, will feed greedily if held to the ewe. In some lambs costochondral abscesses may be palpated along the chest wall or at the sternal region. Abscesses are occasionally encountered /

encountered in the vicinity of the mandible.

The temperature varies considerably and is of little value in assessing the condition of the animal. Death may occur after a period of one to several days as a result of the function of vital structures being mechanically impaired. Nevertheless, many lambs which contract the pyaemic type of disease do not die. In those particular cases the joints are generally found to be affected.

The post-mortem examination of a lamb affected with pyaemia reveals a variety of findings depending on the location of the organisms in the tissues of the body. Thus abscess formation may be present in one or more situations. Externally numerous ticks and tick-bites are usually observed and these are especially noticeable in the axilla and the inner aspect of the hind limbs. They may, however, be found in practically any part of the carcase. On removal of the skin, abscess formation may be found associated with the superficial tick-bites and suppuration may extend into the subcutaneous tissue and underlying muscle groups. The tendon sheaths in the vicinity of joint capsules, in addition to the joints, are favourite sites for the multiplication of the organisms. The main joints affected are those of the limbs and also the costochondral articulations in the thoracic wall. Abscesses in the latter situation frequently produce a localised pleurisy in their immediate neighbourhood. The lungs are rarely found to be affected with /

with abscess formation but the pericardium and myocardium are frequently suppurative. Diaphragmatic abscesses are occasionally observed. The liver appears to be the main organ in which the staphylococci localise. Large single abscesses or numerous smaller lesions may be present in the liver substance. The spleen in some instances contains a few abscesses and the kidneys in many cases are involved in the pyogenic process, both the cortical and medullary substances being affected. Other tissues such as the thymus and adrenal glands have been found to be suppurative in advanced cases of the disease. A detailed examination of the central nervous system often reveals some interesting features. If the dorsal portion of the skull be removed and the vertebral column severed along its entire length an abscess is frequently found in the meninges of the brain or spinal cord. In most cases the abscess can be traced from the muscle group in the vicinity of the vertebrae, between the bony structures of the vertebral column to the coverings of the spinal cord. A considerable area of the meninges may be affected in the thoracic or cervical regions. These lesions explain the occurrence of 'crippled' lambs which have no obvious joint abscesses and which are commonly mistaken for louping-ill cases due to the type of paralysis produced by the suppurative meningitis.

These abscesses despite their varied situations in the body are all equally characteristic. Invariably a considerable /

considerable zone of congestion is present and in the centre of this affected area a yellowish white purulent mass is situated, which escapes readily when the lesion is incised. A certain degree of fibrous encapsulation may be present in those cases which have lived for some weeks after the initial infection.

THE BACTERIOLOGY OF ENZOOTIC STAPHYLOCOCCAL INFECTION.

Since it is clear that this disease is responsible for a considerable mortality and debility among lambs on heavily tick-infested farms, bacteriological studies were undertaken as a preliminary part of a general investigation which had as its object a study of the nature of the disease, the characters of the strains of staphylococcus isolated from affected lambs and immunological and therapeutic measures which might be adopted for its control.

Bacteriologically, the disease has received little attention in the past and it is the object of the present work to describe the cultural reactions of strains of organisms isolated from typical cases of the infection in lambs and to indicate some of their toxicogenic and pathogenic properties.

McFadyean (1894) made the following observations on the causal organism which he isolated from cases of 'pyaemic spinal meningitis':- "This micrococcus has characters which mark it out as a new species. The single organisms are spherical, with a diameter of about 0.3μ, but in the abscesses they are usually present in pairs /

pairs or in irregular groups. It grows rapidly at all temperatures between 70° and 100°F. On slanting agar tubes its cultures have a faint yellow tinge, and the colour is deeper but the growth scantier in the case of potato cultures. On the surface of agar its colonies are flat and nearly circular, with a smooth edge. Single colonies when not crowded too closely attain a diameter of 4 or 5 mm. In very old colonies the centre, when viewed by transmitted light, appears as a dark spot. The growth does not penetrate the agar. It rapidly liquefies gelatin, and deposits at the bottom of the tube a nearly colourless precipitate. At 25°C. the gelatin down to the bottom of the needle track is liquified in three or four days. When cultivated in milk the latter becomes coagulated. In bouillon it grows abundantly, rendering the liquid turbid and depositing a white precipitate. Little or no growth takes place in the complete absence of oxygen. It stains by Gram's method. It excites suppuration when injected under the skin of rabbit or guinea-pig; but only an inflammatory swelling, which disappears without the formation of an abscess in the horse and ox".

In order to obtain more information about the coccus which produces this enzootic infection of lambs, several strains of the organism were studied in greater detail during 1940-42. This work is described in the following /

following sections:-

Section 1. The origin of the strains of staphylococcus and their reactions in various media.

Section II. Coagulase tests and pathogenicity to the mouse.

Section III. Agglutination and cross agglutination reactions of the pathogenic strains.

Section IV. Haemolysin and Toxin Production.

Section V. The experimental infection of the guinea-pig with strain 19L isolated from a natural case of pyaemia in the lamb.

Section VI. The experimental infection of young lambs with strain 19L, together with clinical signs and post-mortem findings.

SECTION I.

THE ORIGIN OF THE STRAINS OF STAPHYLOCOCCI AND THEIR REACTIONS IN VARIOUS MEDIA.

Origin of Strains:

Nineteen strains of staphylococci have been examined in detail during the present investigation. These strains were isolated in the spring of 1940 by Dr W.S. Gordon, Chief Bacteriologist at Moredun Institute, from lambs affected with pyaemia on hill farms in Perthshire. The typing of these strains was commenced by the writer in the autumn of the same year.

The cultures were obtained from various situations such as the liver, limb joints and from abscesses situated in the lower jaw, heart, kidney, spleen, lung and thoracic cavity. They were labelled according to the serial number of the affected lamb and the organ from which the culture was isolated:-

19 L. (Liver)	113 K. (Kidney)
71 L. (Liver)	115 S. (Spleen)
82 L. (Liver)	118 L. (Lung)
84 L. (Liver)	118 H. (Heart)
89 L. (Liver)	132 K. (Kidney)
89 H. (Hock joint)	134 L. (Liver)
90 J. (Jaw abscess)	691 L. (Liver)
100 L. (Liver)	691 K. (Knee)
106 P. (Pericardium)	691 F. (Fetlock)
	691 T. (Thorax)

In three cases cultures were made from more than one site in the same animal.

Reactions on Different Media:

These strains were cultivated on sheep blood agar slopes, and subcultivations were made on this medium at monthly intervals. Films of all the cultures were stained by Gram's stain. All were Gram-positive and showed /

showed the morphological characters usually associated with staphylococci, as described by Topley and Wilson (1941).

Various types of media were employed in order to determine the cultural characters of the strains under examination.

Nutrient Broth. The majority of strains showed similar reactions in this medium. After 24 hours' incubation at 37°C. moderate turbidity and a slight deposit were observed. The turbidity increased on shaking. After a period of two days a surface growth was noticed in the form of a ring round the edge of the tube.

MacConkey's Medium. All the strains produced small pink colonies when incubated at 37°C. for 24 hours and these became somewhat darker in colour after 48 hours.

Agar Plates (Anaerobic). Results were similar in all cases after incubation for 48 hours at 37°C. The colonies were not raised as in other media and were quite colourless. When the cultures were allowed to remain for 24 hours at room temperature, pigmentation appeared, but no true differentiation was possible between the colours of the different strains.

Gelatin. It has been suggested in the past by various workers that gelatin is a useful medium for differentiation between the saprophytic and pathogenic staphylococci. Dudgeon (1908) made use of this medium extensively. /

extensively. The rate of liquefaction and also its extent, is said to be greater with the pathogenic strains than with the saprophytic.

Liquefaction of gelatin was studied both on gelatin plates and in gelatin stab cultures. The results appear in Table I (see appendix) and show that the correlation between the two methods was close. All the strains liquefied gelatin although the degree of liquefaction varied with the individual cultures. 82L produced very definite liquefaction on both types of media. 84L, 89H, and 100L all produced considerable liquefaction. The 691 group yielded poor liquefaction in all cases. The strains 19L, 89L, 113K, 115S, 118L and 118H produced good liquefaction on plates but poor liquefaction in stab cultures. Liquefaction was not marked until after the third day at room temperature (59°F). Subsequent results showed that gelatin liquefaction did not in all cases indicate pathogenicity. For example, 82L, although producing marked liquefaction of this medium, failed to react positively to the coagulase test and was finally typed as a non-pathogenic strain.

Pigmentation. Dudgeon (1908) and other workers regard pigment production as an important aid in typing strains of staphylococci. Accordingly, the chromogenic characters of the various strains were compared on four different types of media, viz. nutrient agar, Leoffler's serum, potato slants, and 33 per cent. milk /

milk agar. The results are shown in Table II.

The temperature and duration of incubation were varied with each medium.

Nutrient agar was found to be of value if the cultures were grown for 48 hours at 37°C. and then allowed to remain for several days at room temperature (59°F).

Leoffler's serum afforded only slight pigment production at room temperature (59°F) but when left at this temperature overnight after primary incubation at 37°C for 48 hours, pigment production was profuse.

Potato slants gave marked colour production provided the cultures were left at room temperature for a considerable time after the initial period of incubation. Periods up to 7 days were required in some cases before the colour became well marked.

Milk Agar. The best results were obtained with 33 per cent. milk agar, Christie and Keogh (1940), on which the pale colonies appeared lighter, and the orange colonies darker, than the surface of the medium. Readings were taken after two days at 37°C and two days at room temperature. The degree of colour production by different strains could be readily compared by spreading a loopful of culture from each strain on the surface of white cartridge paper.

Pigmentation was found to vary considerably according to the medium used, the time of incubation and the temperature. It was quite possible to

obtain /

obtain variations in pigment production by merely altering the environmental conditions.

It will be seen from Table II that the majority of the strains were orange in colour but four strains, namely, 82L, 89L, 90J and 118H were capable of giving rise to only pale yellow-fawn pigment on most of the media employed. It may be noted, however, that these four strains were still capable of producing a slight degree of orange pigmentation if cultivated for 9 days on potato slants. Strain 118H is of special interest because when first isolated it was capable of producing orange pigment on any of the media utilised for chromogenesis, but, after a few subcultivations, it became a pale-coloured strain and lost its pathogenic character.

As a result of the studies on chromogenesis it may be inferred that colour production, though helpful, is not an infallible guide in differentiating pathogenic from non-pathogenic strains of staphylococci isolated fromlambs.

BIOCHEMICAL TESTS

Fermentation Reactions. Considerable work has been done previously regarding fermentation reactions for classifying staphylococci. Winslow et al. (1920) and others have employed sugars for the purpose of typing staphylococci.

The strains at present under examination were grown in a variety of carbohydrates in which Andrade's indicator was used. All the tests were observed for

seven /

seven days as unreliable results may be obtained by readings taken over a shorter period. Each strain was grown in three tubes of each variety of the carbohydrates.

The following fifteen carbohydrates were employed:- lactose, maltose, glycerol, glucose, sucrose, mannitol, salicin, inulin, raffinose, dulcitol, mannose, sorbitol, erythritol, xylose, and galactose. Uninoculated control tubes of the carbohydrates were always maintained under the same conditions of incubation. Table III records the results of these fermentation tests.

No gas was produced in any of these carbohydrates.

All the strains fermented the following sugars:- maltose, glucose, sucrose, mannose and mannitol. No strain had any action on inulin, raffinose, dulcitol, sorbitol, erythritol, and xylose. Galactose was fermented by all strains with the exception of 100L. Lactose was fermented by all strains except 100L. Glycerol was constantly fermented within four days by 82L, 89L, 90J and 118H and a very slight degree of fermentation of this carbohydrate was occasionally produced by the other strains within six to seven days after the commencement of incubation. Salicin was fermented by only four strains - 82L, 89L, 90J and 118H - and it is significant that these strains were the only ones found to be non-pathogenic for the mouse.

It will be noted from Table III that the majority of /

of the strains gave very similar fermentation reactions. Owing to the variable degree of fermentation which may occur, large numbers of sugars must be employed to obtain accurate results and, therefore, carbohydrate fermentation can be considered a somewhat unsatisfactory method of classifying staphylococci.

Litmus Milk. The reactions in litmus milk as indicated in Table IV. The most marked feature was the failure of 100L to act on this medium. The clotting time was variable but the production of acid and eventual clotting was constant for all the other strains examined with the exception that 118L produced acid but did not clot the milk. The clots generally contracted after a few days incubation, resulting in the expression of whey. Previous workers generally agree that strains of Staphylococcus aureus form acid and clot in litmus milk. e.g. Minett (1936).

FURTHER BIOCHEMICAL TESTS

The results are shown in Table IV.

Methods and results:-

Methyl Red Test. Five drops of 0.04 per cent. solution of methyl red were added to a 3-day culture of glucose phosphate medium (peptone 0.5 g. K_2HPO_4 0.5 g. glucose 0.5 g., H_2O 100 c.c., pH 7.5) at 37°C. All the strains examined were methyl red positive. It is generally believed that a positive reaction to the methyl red test is given by all staphylococci except the citreus species, Topley and Wilson (1941).

Voges /

The majority of staphylococci reduce methylene blue and all the strains under examination gave positive results.

Indole. Indole production was tested by the method of Holman and Gonzales (1923). None of the strains examined formed this substance.

Hydrogen Sulphide. The production of H_2S was tested on lead acetate medium, which is a heart extract broth containing 4 per cent. peptone, 2.5 per cent. agar and an equal quantity of sterile 0.1 per cent. solution of basic lead acetate. Andrewes and Gordon (1905/6) found that a small quantity of hydrogen sulphide was formed by the pyogenic cocci. None of the strains at present under investigation produced hydrogen sulphide.

These biochemical tests were suitably controlled by using cultures of organisms of known reaction so as to determine if the reagents employed were capable of producing satisfactory positive or negative results according to the culture employed.

SECTION II.

COAGULASE TESTS AND PATHOGENICITY TO THE MOUSE.

Coagulase Test.

The ability of strains of staphylococci of human origin to coagulate citrated plasma has been studied by numerous workers in recent years and Cruikshank (1937) has shown the coagulase test to be of considerable value in differentiating between pathogenic and non-pathogenic types.

Methods.

In order to obtain the plasma, equal quantities of blood and 4 per cent. sodium citrate solution were mixed and centrifuged. Thereafter the supernatant fluid was removed.

The cultures were grown on plain agar slopes for 24 hours at 37°C. and the growths emulsified in normal saline. One loopful of each suspension was added to 0.5 c.c. of plasma. Readings were taken at 3, 5 and 24 hours, the tubes being incubated at 37°C. The following types of plasma were employed:- Human, rabbit, sheep and horse, since it was considered that there would probably be a certain degree of variation in the coagulating effect of the organisms on different varieties of plasma.

Results.

Table V shows the comparison between the four coagulase reactions.

The best results were obtained with the plasma from the /

the human, sheep and rabbit, suitable readings being obtained after an interval of three hours whereas the horse plasma was considerably slower in reacting to the presence of the organisms, overnight readings being the most suitable.

The results obtained showed a close correlation with the pathogenicity and toxicity tests. All the strains examined gave positive coagulase tests in all four varieties of plasma with the exception of 82L, 89L, 90J and 118H. These four strains have subsequently proved to be of a non-virulent nature and have been classified as coagulase-negative strains. The strain 118H is of interest in that it produced a positive reaction to rabbit plasma in the first instance, but subsequently, after a few sub-cultures failed to react either to rabbit plasma or to any other type which was employed.

The pathogenicity and toxicity tests showed the coagulase reaction to be of great value in the classification of the various strains. The types of staphylococci could clearly be divided into two main groups irrespective of their colour and fermentation reactions, namely, a coagulase-positive group comprising the majority of the lamb strains and a small group consisting of the 4 coagulase-negative types.

These coagulase-negative strains have shown themselves to be incapable of causing death in mice, or

of giving rise to the production of either α or β toxin in various media.

Apart from animal inoculation tests the coagulase reaction is probably the most satisfactory method of determining the pathogenicity of strains of staphylococci.

PATHOGENICITY TO THE MOUSE

The nineteen strains under examination were tested for pathogenicity to the mouse.

Experimental animals.

Young mice of both sexes approximately 20 g. in weight were employed.

Preparation of inoculum.

The cultures were grown on plain agar slopes overnight at 37°C. aerobically so as to avoid the presence of toxins in the resulting suspension.

Each strain was emulsified in normal saline and standardised to an opacity matching No.8 tube on Brown's scale. The mice were then inoculated with varying amounts of staphylococcal suspension. The volume of the inoculum was maintained constant at 0.5 c.c. with normal saline irrespective of the quantity of suspension of organisms present.

Methods.

By preliminary tests which consisted of inoculating quantities of standardised suspensions intraperitoneally, intravenously and subcutaneously, the strains 82L, 89L, 90J and 118H were proved to be non-pathogenic. The non-pathogenicity of these four strains correlated perfectly /

perfectly with their coagulase reactions which have already been described.

Three groups of mice were inoculated with the remaining fifteen strains. Group A was inoculated by the intravenous method, Group B by the intraperitoneal route and Group C subcutaneously. The results are shown in Table VI, A, B and C. Three mice were used for each strain and the doses ranged from 0.1 c.c. to 0.00001 c.c. of standardised suspension, except in the case of Group C, where doses of 0.3 c.c., 0.1 c.c. and 0.01 c.c. were employed and two mice were inoculated with each dose.

Results.

A. Intravenous Group

The intravenous method of inoculation constantly produced typical pyaemic changes depending on the quantity of organisms employed. The animals lived for a considerable time after infection and the pathological changes in the various organs closely resembled the lesions encountered in natural cases of pyaemia in lambs. The kidney tissue appeared to be the principal site of infection, the lesions consisting of numerous pyogenic foci. Costochondral and myocardial abscesses were noted in most cases. The liver substance was found to be infected on three occasions.

B. Intraperitoneal Group

The mice appeared to be much more resistant to this method of inoculation than to the intravenous route. Those /

Those mice which died as a result of intraperitoneal inoculation succumbed soon after infection, generally at one to three days. The post-mortem appearance of each mouse indicated that death had resulted from the proliferation of organisms in the peritoneal cavity and the absorption of toxins elaborated by these staphylococci. The only constant macroscopic lesion observed was an extensive suppurative peritonitis, from which the organisms could be readily isolated in pure culture. Occasionally abscesses were present on the visceral surface of the liver.

C. Subcutaneous Group

Mice were inoculated subcutaneously because the pyaemic infection of lambs apparently originates from the staphylococci gaining access to the subcutaneous tissues. The results of this pathogenicity test indicated that a few strains which were lethal to mice when introduced intravenously were incapable of producing death when inoculated subcutaneously. The deaths which occurred as a result of this method of inoculation appeared to be produced mainly by rapid abscess formation at the site of inoculation with the subsequent absorption of toxins. Pyaemic lesions were entirely absent from all the infected animals. The only lesion observed at autopsy was abscess formation, varying in severity, and considerable necrosis, at the inoculation site.

Attempts were made to cultivate the organisms from blood /

blood obtained during life from the tail veins of the mice in all these groups. On no occasion was a positive culture obtained from any mouse in the intraperitoneal or subcutaneous groups, but mice from the intravenous group yielded pure cultures at irregular intervals. This result was to be expected, since in the natural course of the disease in the lamb, the organisms rapidly leave the systemic circulation, localise in the organs and joints and produce bacteraemia at irregular intervals rather than a continuous septicaemia.

Pathogenicity tests in mice invariably presented some considerable difficulty. The preliminary standardisation of the suspension by means of the opacity tubes gave only an approximate number of cocci per c.c. owing to the characteristic clumping of the organisms and plate counts of dilutions of the same suspension could not always be relied upon to give identical figures. Therefore, the doses employed were spaced at considerable intervals. Moreover, it will be observed that mice which received the same quantity of inoculum often died at irregular periods after infection. This probably resulted from the capacity of the staphylococci to localise in different tissues in different animals, especially in the more chronic type of the disease. In the acute septicaemic cases produced by the inoculation of larger doses, the variation /

variation in the time of death was not so marked.

All the mice employed in these pathogenicity tests were kept under observation for one month after inoculation so as to detect any delayed chronic type of infection.

These preliminary pathogenicity experiments agreed perfectly with the coagulase reactions since all the coagulase-positive strains were proved to be pathogenic by one or more of the inoculation routes employed.

S E C T I O N III.

AGGLUTIONATION AND CROSS-AGGLUTINATION REACTIONS OF THE PATHOGENIC STRAINS.

These tests were carried out in order to determine the serological relationship between the various pathogenic strains.

There is little information in the literature relating to the agglutination reactions of staphylococci of animal origin. It has usually been accepted that strains from the pathogenic sources are agglutinated by sera made from such strains and that sera from saprophytic organisms will not agglutinate pathogenic types.

Methods.

Immunisation of rabbits for agglutination and cross-agglutination tests

A number of 48-hour cultures at 37°C. on agar slopes were emulsified in normal saline, 8 c.c. being added to each culture. 0.25 per cent. formalin was added to each suspension and the tubes were incubated at 37°C. Subcultures were made in order to determine when the cultures were inactivated. Inactivation occurred at 18 hours. The rabbits received nine intravenous injections of the formalinised cultures according to the scheme of Cowan (1939). Samples of sera were obtained before the first inoculation and also 7 days after the nine inoculations were completed. These sera were tested for agglutinins against their respective antigens and cross-agglutination tests were also /

also performed.

Preparation of suspensions of organisms for agglutination tests.

A quantity of 5 c.c. of normal saline was added to a 24-hour culture of the organisms on a plain agar slope. This suspension was standardised to Brown's tube No.6. The supernatant fluid was then decanted into sterile tubes.

Method of preparing serum dilutions.

Twelve tubes were used for each test. 0.1 c.c. serum was added to 1.4 c.c. normal saline in mixing tubes. No.2 tube and each of the successive tubes received 0.7 c.c. of saline. 0.7 c.c. of the diluted serum from the mixing tube was added to tubes 1 and 2. From No.2, 0.7 c.c. was taken and placed in No.3 tube, etc. 0.7 c.c. was withdrawn from the last tube. The antigen consisted of 0.7 c.c. of the particular organism under consideration and this was added to each tube separately. The tubes were incubated at 37°C. overnight when readings were made. When necessary the sera were preserved by the addition of 0.25 per cent. $CHCl_3$.

Results.

As can be seen from Table VII, all the sera produced from rabbits inoculated with the pathogenic strains, agglutinated their corresponding antigens, and in addition, agglutinated the other pathogenic types with the exception of 71L. The serum prepared from this strain /

strain would only produce agglutination of strains 71L and 69L.

As a result of these tests there appears to be a considerable degree of serological relationship between the various strains of coagulase-positive staphylococci isolated from lambs.

SECTION IV.

HAEMOLYSIN AND TOXIN PRODUCTION.

Glenny and Stevens (1935) described two toxins produced by staphylococci, namely α and β types. In order to determine which type of toxin was produced by strains of staphylococci isolated from lambs a series of haemolytic, lethal and necrotic tests were carried out.

HAEMOLYSIS ON BLOOD AGAR.

All the 19 strains were examined for haemolytic activity on blood agar plates. Five per cent. blood agar was employed and haemolytic activity tested on rabbit, sheep and horse blood. The last gave inferior results and only a few strains produced haemolysis on this medium. Cultures of the organisms were grown overnight in nutrient broth at 37°C. A loopful of each culture was dropped on to the plate according to the method of Bryce and Rowntree (1936), one plate sufficing for the examination of four strains.

Results.

The α haemolysis was indicated by a narrow clear zone round the colony. The β type of Glenny and Stevens was characterised by a zone of partial haemolysis in sheep blood in which the blood was darkened in colour. After overnight refrigeration the darkened zone cleared considerably. The $\alpha\beta$ type consisted of a combination of these two varieties of haemolysis, the α haemolysis giving rise to a clear zone near the colony and the β haemolysis /

haemolysis appearing as a partially haemolysed zone at the periphery of the haemolytic area. As will be seen from Table VIII, the majority of the strains showed the α β type of haemolysis. Some strains, namely, 82L, 89L, 90J, and 118H, produced no haemolysis. It is of some interest that 71L and 69IF produced haemolysis in sheep blood but failed to produce any reaction in rabbit blood. This is characteristic of strains producing mostly β haemolysis. That these two strains do in fact produce mostly β toxin is shown later in the paper since neither toxin caused necrosis on the skin of depilated guinea-pigs nor were they lethal to mice when injected intravenously.

TOXIN PRODUCTION

Methods.

(a) 0.3 per cent. agar. The medium consisted of 0.3 per cent. agar broth inoculated from fresh broth cultures incubated overnight at 37°C.

Five plates were used for each culture and they were incubated for sixteen hours at 37°C. in an atmosphere of 20 per cent. CO_2 and 80 per cent. O_2 approximately. The agar was then ground up in a mortar and filtered through gauze and finally through a filter composed of keiselguhr and paper.

Burnet (1930) used 1 per cent. agar for the preparation of staphylococcal toxin, but in our experience 0.3 per cent. agar gave similar results and allowed /

allowed small quantities of media to be filtered effectively.

(b) 0.3 per cent. agar with the NaCl content replaced by $MgSO_4$. The technique was similar to the above.

(c) Parker's Medium. Several modifications of Parker's Broth, incorporating semi-solid agar medium were also employed. The potency of the toxins so produced was similar to that of the toxins obtained from the ordinary semi-solid medium.

(d) Walbum's Medium. The standard Walbum medium was used and the following technique adopted:-

Fresh 16-hour broth cultures of the organisms were used for the inoculation of the Walbum's medium. These broth cultures were standardised to No.6 Brown's tube and 0.2 c.c. was used as the inoculum. The Walbum's medium, 8 c.c. foreach culture, was incubated at 37°C. in an atmosphere of 20 per cent. CO_2 and 80 per cent. O_2 approximately. The CO_2 content was renewed every second day. The cultures were centrifuged and the supernatant fluid pipetted off. This constituted the toxin.

(e) Chick Embryo Medium. This medium consisted of Tyrode's solution and 10-day old minced chick embryos. The cultures were incubated for various periods aerobically and anaerobically.

The crude toxins produced by these methods were tested for:-

a) /

a) Haemolysin production by tests similar to Bigger's method (1933) in which descending dilutions of toxin were mixed with 1 per cent. sheep red blood corpuscles.

b) Lethal effect in mice by intravenous inoculation. The inoculum was maintained constant at 0.5 c.c. irrespective of the quantity of toxin present.

c) Necrotic action on the skin of depilated guinea-pigs which were injected intradermally with 0.1 c.c. of varying dilutions of toxin.

Suitable controls were used in all these experiments.

Various methods of enhancing toxin production have been investigated such as varying the incubation time both of the broth inoculum and of the various final media and also by altering the CO_2 content of the atmosphere.

Results.

The best results have been obtained in Walbum's medium and the optimal period for toxin production has been shown to be approximately nine days. Table IX depicts the results obtained with typical batches of toxin prepared in this medium. Four of the nineteen strains examined, namely, 82L, 89L, 90J and 118H, failed to cause haemolysis of 1 per cent. sheep red blood corpuscles in vitro, failed to produce necrosis of the skin in guinea-pigs and were not lethal to mice.

Strains 71L and 691F produced a marked 'hot cold' lysis /

lysis *in vitro*, practically all the lysis occurring after the tubes had been removed from the incubator; they also produced only a faint flushing in the skin of a depilated guinea-pig in a dose of 0.1 c.c.

The remaining thirteen strains all gave high haemolytic titres. These high titres were due principally to the β content of the toxins, since the haemolysis was markedly increased after refrigeration. They also produced necrosis of the skin in guinea-pigs and were lethal to mice. The majority of these thirteen toxins gave very similar results. With certain batches of toxins grown on Walbum's medium, strains such as 19L, 106P and 115S killed mice in a dose of 0.0125 c.c. intravenously. Other strains killed regularly with 0.025 c.c.

The smallest quantity of toxin which would produce skin necrosis in the guinea-pig was 0.00625 c.c. of strain 19L or 115S.

In the other media, toxin production was not so marked and high haemolytic titres were not obtained. Marked skin necrosis in guinea-pigs and lethal effects in mice were not readily produced.

SECTION V.

THE EXPERIMENTAL INFECTION OF THE GUINEA-PIG WITH STRAIN 19L ISOLATED FROM A NATURAL CASE OF PYAEMIA IN THE LAMB.

The following experiments were undertaken to determine if a disease similar to that which occurs naturally in lambs could be produced in the guinea-pig. The majority of writers who have investigated the pathogenicity of staphylococci of human origin have been interested mainly in the production of septicaemia, the rabbit or the mouse being used as the principal experimental animal. The pyaemic condition is sometimes referred to and in most cases is mentioned as an unexpected sequel to the apparent resistance of an animal infected with a suspension of pathogenic staphylococci.

In order to investigate the production of pyaemia, varying amounts of organisms were injected subcutaneously, intraperitoneally and intracardially in small groups of guinea-pigs. Those inoculated subcutaneously developed only abscesses at the inoculation sites. The animals which were inoculated intraperitoneally remained healthy during the observation period of one month, whereas payemia developed in the group inoculated intracardially. This pyaemic condition was the subject of further investigation which may be conveniently described under two heads.

A) The determination of a suitable infective dose of strain 19L for the guinea-pig, including symptoms and post-mortem lesions produced.

B) /

B) The pathogenesis of the infection and the propagation of the organisms in the tissues of the body.

A. THE DETERMINATION OF A SUITABLE INFECTIVE DOSE OF STRAIN 19L FOR THE GUINEA-PIG INCLUDING SYMPTOMS AND POST-MORTEM LESIONS PRODUCED.

Technique

Experimental Animals.

Guinea-pigs of both sexes varying in weight from 500 to 700 g. were employed. All their sera were negative to the agglutination test against strain 19L prior to inoculation.

Source of strain 19L

As described in Section 1 dealing with the typing of staphylococci isolated from lambs, strain 19L was isolated in the spring of 1940 from the liver substance of a lamb affected with the pyaemic type of staphylococcal infection. It possessed the typical characters of the lamb strains of staphylococcus and had been maintained on sheep blood agar medium since its original isolation. At the commencement of this experiment the culture had been subcultivated on twenty-nine occasions, the subcultures having been made at monthly intervals.

Suspension of Organisms

The organisms were grown on plain agar slopes for eighteen hours at 37°C. aerobically. A suspension of the cultures was prepared in normal saline and standardised to an opacity matching that of No.8 tube on Brown's scale.

Method /

Method of Inoculation

The animal was stretched tightly on its back and the injection was made directly into the heart by means of a 1 c.c. glass syringe and a 22 S.W.G. needle. No anaesthetic was employed and each animal received a total volume of 1 c.c. although the actual quantity of bacterial suspension varied from 1 c.c. to 0.0015 c.c.

All the animals were retained under observation for one month in order to detect the possible development of a chronic infection.

Method of detecting the presence of staphylococci in the tissues.

When it was necessary to determine if staphylococci were present in an organ, the following procedure was adopted:- Smears were made from the tissues and stained by Gram's stain. Thereafter cultures were made upon 5 per cent. sheep blood agar plates and incubated overnight at 37°C. aerobically. The plates were then placed in the refrigerator for a few hours prior to examination in order to facilitate reading the type of haemolysis. Coagulase tests using rabbit plasma were employed in the identification of pathogenic staphylococci.

Experimental

A series of guinea-pigs were inoculated intracardially with the previously described suspension as indicated in Table X. The macroscopic lesions produced are shown in Table XI.

The /

The time from inoculation until death varied in different animals according to the dose employed. Rapid death followed the injection of quantities of suspension of 0.1 c.c. or more. One of the 0.05 c.c. subjects survived for a period of 3 weeks. A week after inoculation this animal was showing typical cachexia but no lesions were present either in the joints or in the wall of the thoracic cavity. 0.025 c.c. and 0.0125 c.c. killed regularly within 5 days, though some degree of variation in the time of death could be expected. With an inoculum of 0.0063 c.c. a very mild type of disease was produced in two cases. If the animal which had been inoculated with this amount or a smaller dose, survived over a period of eight days, recovery of the guinea-pig occurred, although clinical evidence of the presence of a suppurative focus might appear. In one case a large abscess developed in the costochondral region of the chest wall and in another a suppurative arthritis occurred in the left elbow joint. Both these lesions were still clinically palpable three months after infection. It is of interest that, like the natural disease, animals which had contracted the more chronic form of the condition ultimately recovered, although the lesions persisted for a considerable time in each case. As a result of this experiment it was decided that a suitable infecting dose for the production of pyaemia in the guinea-pig was 0.0125 c.c.

Symptoms

Animals /

Animals receiving doses ranging from 0.05 c.c. to 0.0063 c.c. developed a chronic type of disease. This was characterised by considerable loss of bodily condition. About the second to the third day after infection many animals showed incoordination of gait. Those which received a heavy infective dose developed a more acute infection followed by death before any definite pyaemic lesion had developed. This corresponds with the acute type of the disease which occurs naturally in lambs. Guinea-pigs affected with the acute disease frequently showed muscular tremors, particularly obvious in the hind limbs. These symptoms of trembling and incoordination are suggestive of infection of the central nervous system and the development of such symptoms in lambs, in the absence of pyaemic lesions at autopsy, makesit difficult, if not impossible, to differentiate between this condition and louping-ill without recourse to cultural and biological tests.

Post-mortem Findings.

In general the findings were similar in each case except for the fact that the larger the infecting dose the smaller the number of visible abscesses. The parenchymatous organs usually showed evidence either of congestion or abscess formation. On only two occasions was the liver affected to the extent of suppuration - once when the inoculum was 0.1 c.c. and once /

once at 0.0125 c.c. In one case when 0.0125 c.c. was injected the kidneys were entirely unaffected and cultures made from these organs proved negative. Moreover, no visible kidney lesions were present microscopically. In this animal, however, numerous small abscesses were present throughout the lung.

It could usually be anticipated that definite abscess formation would be found in the kidneys if the animal survived longer than 48 hours. Even at an earlier stage of the infection very small pin-point abscesses could occasionally be seen macroscopically. Sections were prepared from kidneys affected with typical lesions and stained by haematoxylin and eosin and Gram's stain. Microscopically they showed typical pyaemic nephritis with multiple foci of organisms each surrounded by a considerable zone of infiltrating leucocytes. Each kidney section was very vascular and extensive areas of both cortical and medullary substances showed degenerative changes and necrosis. The glomeruli appeared to be particularly involved in the suppurative process. Dense masses of staphylococci could be observed in sections stained by Gram's stain.

In most cases the liver and spleen were markedly congested and subsequent bacteriological examination showed that these organs contained a heavy concentration of organisms.

When a heavy infective dose was given and death occurred rapidly, there was excess of pleural and pericardial /

pericardial exudates. The exudates were usually blood stained and slightly fibrinous. Pleurisy varying in severity and multiple abscess formation in the heart wall, lungs, costochondral ariticulations and the diaphragm were frequently observed.

No involvement of the limb joints was found in any animal subjected to post-mortem examinations within five days of its receiving an infective dose.

Bacteriological Examination.

Smears from the tissues of the guinea-pigs stained by Gram's stain were usually sufficient to indicate the presence of large numbers of staphylococci. If there was congestion of an organ without a focus of suppuration the tissue was inoculated on blood agar plates and haemolytic colonies were picked off, identified morphologically and tested for coagulase production.

The heart blood of each animal examined, invariably yielded a pure culture of staphylococci whichwere haemolytic and coagulase-positive. Any organ which showed the slightest evidence of macroscopic abnormality produced a copious growth of typical Staphylococcus aureus when subjected to cultural examination.

B. THE PATHOGENESIS OF THE INFECTION AND THE PROPAGATION OF THE ORGANISMS IN THE TISSUES OF THE BODY.

Technique

Eighteen guinea-pigs, varying in weight from 500 to 700 g. /

700 g. were tested for the presence of agglutinins for strain 19L. All were negative. These animals were inoculated intracardially with 0.0125 c.c. of a suspension of strain 19L prepared as indicated in Part A. of this section.

At intervals of 1, 2, 4, 8 and 48 hours after infection, three guinea-pigs were killed and the last three allowed to die. Death occurred five days after infection. At the time of death blood was collected and diluted to 10 per cent. in normal saline. The spleen, liver, submaxillary lymphatic gland, precrural lymphatic gland and kidney were removed with sterile precautions, weighed and pulped in separate mortars and sufficient normal saline was added to make a 10 per cent. suspension of each tissue.

Tenfold dilutions were made giving a range of dilutions from 10^{-1} to 10^{-10}. 0.5 c.c. of each dilution was inoculated on to a five per cent. sheep blood agar plate, since this amount was found to conveniently cover the area of the petri dish. Thus ten plates were used for the examination of each organ. These were incubated as indicated in Part A of this section.

Results.

The average degree of infection in each organ is indicated in Chart I. (see appendix). The various stages in the development of the disease could be ascertained by examining the tissues of the animals at /

at different intervals. Little work has been done concerning the actual propagation of the staphylococci in the tissues of the body. The majority of writers have studied only the final pathological picture after the death of the animal. Burnet (1929), working with staphylococci of human origin, inoculated a rabbit with a staphylococcal suspension, and determined the course of the ensuing septicaemia by taking blood samples at intervals from the ear vein and plating them upon blood agar medium. Many writers including Burnet, record the occurrence of pyaemic nephritis associated with a staphylococcal infection. It was felt that this study of the progressive development of the condition might assist in providing a more detailed outline of the course of the infection. It will be seen from Chart I that the primary phase of the disease was characterised by a concentration of organisms in the liver and spleen - those organs being intimately connected with the reticulo-endothelial system. The blood also contained numerous staphylococci during the first few hours after infection. The filtering capacity of the liver and spleen persisted for a considerable time although the concentration of organisms in the blood slowly diminished. Towards the termination of the disease the multiplication of cocci progressed in the kidney tissue so that the kidneys eventually became the main foci of the pyaemic distribution /

distribution of the organisms. The colony counts from the brain probably indicated the degree of infection present in the meninges and cerebral vessels, since these two tissues were included in the portion of brain taken for examination. The lymphatic glands which were examined, namely, the precrural and submaxillary, showed a considerable variation in the degree of infection in different animals.

The most striking feature was the rapid propagation of the staphylococci in the kidney tissue after eight hours. A most characteristic agonal multiplication was observed in all the main tissues of the body at the time of death, all the colony counts showing a marked increase compared with those recorded at 48 hours.

The colony counts varied according to the individual animal, e.g. in one guinea-pig which died at five days the submaxillary lymphatic gland gave a higher figure than would normally have been expected for this organ. This was due to the fact that a small abscess had developed in that particular region. It is interesting to note that the precrural gland also gave an unusually high count in this particular instance although no visible lesion was present. Unfortunately when working with staphylococci as indicated in the mouse pathogenicity tests, it is not possible to obtain the same even suspensions of organisms such as can be obtained /

obtained with other bacteria e.g. Brucella. The cocci are invariably present in their characteristic clumps and thus the subsequent colony counts on plates inoculated with the material may show some considerable degree of variation. Indeed, a series of plates seeded from one original suspension may give varying counts. Moreover if given quantities of staphylococci are injected into a series of guinea-pigs these animals may react to the presence of the organisms in a variety of ways according to their individual susceptibility. Thus, in one guinea-pig, the predilection sites of the organisms may be the heart wall and costochondral articulations, in another, the kidneys may be principally affected. This phenomenon is even more marked in young lambs in which the organisms localise in widely different areas.

Despite these difficulties, it may be appreciated from this experiment that the blood colony counts steadily diminished and that the principal site for the multiplication of the organisms was the kidney tissue, after the initial concentration in the liver and spleen.

SECTION VI.

THE EXPERIMENTAL INFECTION OF YOUNG LAMBS WITH STRAIN 19L TOGETHER WITH CLINICAL SIGNS AND POST-MORTEM FINDINGS.

As a result of the pathogenicity tests in mice and guinea-pigs it was decided to attempt to produce the typical staphylococcal disease in lambs by intravenous inoculation.

The number of animals available did not permit the accurate determination of the smallest infective dose of staphylococcal suspension for the young lamb and therefore, this preliminary work was confined chiefly to a study of the experimental production of the disease, together with the clinical signs associated with this condition and also the bacteriological and pathological findings.

ANIMALS USED

Number	Breed	Sex	Age in Days	
424	Cheviot	F.	1	
425	"	M.	2	
428	"	F.	6	
430	"	F.	13	Twins
431	"	M.	13	Twins
444	"	M.	27+	
453	"	M.	14	+ Twins
454	"	M.	14	+ Twins
455	"	M.	14	

+ Signifies lambs born from ewes which had received staphylococcal toxoid prior to lambing. The ewe which gave birth to lamb No.444 had been inoculated subcutaneously with 5 c.c. of toxoid on two occasions prior to lambing. Lambs No.453 and 454 were also born from an inoculated ewe, eleven subcutaneous inoculations, /

inoculations, each consisting of 5 c.c., having been administered prior to parturition.

Origin and preparation of the staphylococcal suspension.

Strain 19L was employed in the following experiment. The suspension for the inoculation of the lambs was prepared in the same manner as for the guinea-pig pathogenicity tests described in section V.A.

Method of Inoculation

The lambs were inoculated with varying amounts of suspension in an endeavour to produce the characteristic staphylococcal infection. All the inoculations were made intravenously, the jugular vein being the selected site in each case. Prior to inoculation, the skin over the vein was prepared asceptically, the wool being closely clipped and tincture of iodine applied. The volume of inoculum was constantly maintained with normal saline at 1 c.c., although the quantity of suspension of organisms varied.

After inoculation the temperature and also the clinical appearance of each animal was noted at frequent intervals and in some cases colony counts were made during life from samples of blood obtained from the jugular veins of the lambs.

Cultural Methods.

For the purpose of detecting the presence of organisms in the systemic circulation of the affected animal after inoculation, samples of blood were /

were withdrawn from the jugular vein by means of a sterile 1 c.c. syringe. 0.1 c.c. of blood was mixed with 0.9 c.c. normal saline and from the resulting mixture a series of ten-fold dilutions was prepared. 0.5 c.c. was removed from each dilution and spread upon a 5 per cent. sheep blood agar plate. These plates were incubated as described in section V.A. The method employed for estimating the number of organisms present in organs at autopsy has been described in section V.B. The following organs of lambs affected with experimental pyaemia were examined by this method:- lung, liver, spleen, kidney, submaxillary lymphatic gland, and precrural lymphatic gland.

Results.

Since two distinct types of staphylococcal infection were produced, namely, an acute condition and also a more chronic pyaemic type of the disease, it is convenient to describe these two varieties separately.

THE ACUTE FORM OF STAPHYLOCOCCAL INFECTION.

Experimental

Two animals Nos. 424 and 425 were inoculated with the suspension prepared by the method which has been previously described. These two lambs received intravenous inoculations of 1 c.c. and 0.1 c.c. respectively. /

respectively.

Clinical Signs.

There was little to be observed until two to three hours after inoculation when the lambs appeared listless, dull, and disinclined to move about with the ewes or to attempt to suck. They were observed to lie down frequently and from time to time they appeared to have difficulty in rising. There was considerable variation in the respiratory rate, the animals breathing rapidly for a few minutes and then again returning to their normal rate of respiration. The respiratory rate increased rapidly when the animals were forced to move or if they were handled. The temperatures of both lambs rose slightly after a period of a few hours, but there was no sustained rise throughout the course of the infection. Just prior to death the temperatures in both cases dropped to subnormal. Lamb No. 424 showed some interesting symptoms. At about 20 hours after infection muscular tremors could be seen in various groups of muscles especially at the shoulder region. Salivation was profuse and the animal neither showed any inclination to suck nor did it attempt to rise when approached. At this stage the temperature had dropped to 103°F., the highest temperature recorded being 104°F., two hours previously. Lamb 425 showed similar signs to 424 except that definite knuckling at the fetlocks and knee-joints occurred at 24 hours. No. 424 died approximately 25 hours after inoculation whereas /

whereas No. 425 survived for a further period of 6 hours. The close similarity to certain phases in the clinical syndrome of louping-ill was noted in both these lambs at various stages of the infection. Blood cultures obtained during life from each case were positive at 24 hours but no colonies were detected prior to this although samples of blood were withdrawn at frequent intervals. The macroscopic changes involving the organs of both lambs are recorded in detail in Table XII. The microscopic lesions observed in the various tissues indicated typical toxic changes, and the kidneys appeared to be involved in the preliminary stages of abscess formation, much congestion and considerable infiltration of polymorphonuclear leucocytes being present.

Cultures of the various organs were made at autopsy. As will be seen from Table XII the majority of the organs or fluids examined gave positive cultures from each lamb.

The type of condition produced experimentally in these two animals was suggestive of a toxaemia in the early stage of the infection followed by a bacteraemia just prior to death, and it is probable that the majority of the organisms which were inoculated were held in the main filtering organs of the body, such as the lungs, liver, spleen and kidneys, in which positions they proliferated and elaborated toxin. The organisms were /

were only to be found in the blood stream by cultural methods shortly before death and even then only a few colonies were observed.

Numerous sections were prepared from the tissues of both lambs and stained by haematoxylin and eosin and Gram's stain. The usual changes associated with toxaemia were noted in most organs but, of the lesions present, none could be described as being specific for this type of disease alone. It is interesting to note that a massive staphylococcal infection of the liver and spleen was present despite the fact that these organs did not show any marked change macroscopically. Sections of these tissues stained by haematoxylin and eosin did not indicate any notable involvement of the liver or spleen tissue beyond the commencement of degenerative changes and the presence of infiltrating leucocytes. By Gram's stain, however, masses of cocci could be discerned in both the liver and spleen substance, especially in the latter organ.

From the work detailed above it will be readily appreciated that acute staphylococcal infection in young lambs produces no characteristic symptoms or lesions which may be of value in recognising the condition by clinical or macroscopic examination.

In the two animals which were infected in this experiment, staphylococci were difficult to obtain from the blood during life and although numerous samples were withdrawn, it was not until just prior to death /

death that a positive culture was procured. Thus, there is little possibility of diagnosing the condition in the field by blood cultural methods, since death would probably ensue as a result of the toxaemia, before a blood sample could be obtained.

As a result of this preliminary work, we were able to conclude that an acute form of staphylococcal infection could be produced in the young lamb, that blood colony counts could only indicate infection at certain phases of the disease and that the macroscopic post-mortem findings were in themselves not sufficiently characteristic to enable a diagnosis to be made on these grounds alone.

THE PYAEMIC TYPE OF STAPHYLOCOCCAL INFECTION

Experimental.

Seven animals were employed. These lambs were Nos. 428, 430, 431, 444, 453, 454 and 455. All these animals received 0.001 c.c. of standardised staphylococcal suspension intravenously except Nos. 428 and 430 which received doses of 0.01 c.c.

Clinical signs.

A few hours after inoculation, all the lambs appeared disinclined to move. They were observed to lie about and they were not anxious to follow the ewes. Gradually the lambs became more unwilling to exert themselves to any extent and at 24 hours definite symptoms appeared. These usually consisted of some manifestation of the formation of joint lesions, congestion /

congestion of the visible mucous membranes, increased respiratory rate and a febrile reaction. The symptom which was most marked was the impairment of joint movement. In all the lambs there was evidence of incoordination of gait, either at 24 hours or shortly afterwards. This could be attributed either to the joint lesions or to the involvement of the central nervous system in the suppurative process. This inability of the lambs to progress in their normal manner was of special interest because of the common history given by stock owners and shepherds, namely, "the lambs were stiff and did not follow the ewes readily". In fact the lambs at this stage showed symptoms which were indistinguishable clinically from early infection with louping-ill. The variation in the respiratory rate of the affected lambs was not constant. It may be stated, however, that the respirations increased a few hours prior to death, when they sometimes assumed almost a 'gasping' character. The appetite was not markedly altered and in all cases the lambs continued to feed although not so eagerly as previously. The temperatures as will be seen from Chart II usually increased a short time after inoculation and in some lambs a sub-normal temperature was recorded just prior to death. At the height of the febrile reaction about 24 hours after inoculation, a blood sample withdrawn from the jugular veingave a positive culture in some cases as is indicated in Table /

Table XIII, but it will be readily appreciated that a negative culture did not indicate the clinical condition of the animal. Usually at varying periods before death, according to the individual animal, a comatose condition intervened and each lamb was noticed to salivate profusely. At this stage 'trembling' could be discerned, practically all the superficial musculature being affected. In one case, lamb No. 428, there was profuse diarrhoea in the later stages of the infection.

The main clinical signs are indicated in detail in Table XIV.

Macroscopic Findings at Autopsy.

The post-mortem findings in the lambs were very similar and may be briefly indicated as follows:- The liver and spleen were sometimes congested but apart from this congestion little change was apparent in either of these organs except for the fact that the latter organ was definitely enlarged in lamb No. 431. The kidneys were either very congested or involved in abscess formation in every lamb examined. The vessels in the mucosa of the bladder appeared distended and the urine was cloudy in appearance in all cases. In lamb No. 428 the mesenteric glands were enlarged and there was slight excess of peritoneal fluid present. Small areas of pleurisy were usually found in the thoracic cavity at the costochondral articulations. Congestion and/or abscess formation was evident in all the lungs examined. Pericarditis with involvement of the epi-myo-and endocardium, was a common feature of the disease. In five of the seven animals, lesions were found /

found encroaching on the nervous tissue or pressing on the meninges. These lesions were usually associated with the periosteum of the surrounding bone. Abscesses were present in the subcutaneous tissue and superficial muscles of four of the lambs. Limb joints such as the fetlock, knee, elbow, stifle, and shoulder joints were affected in many instances. Cultures could often be obtained from joints which presented no macroscopic abnormalities.

A detailed account of the post-mortem and cultural findings in the pyaemic cases is given in Table XV.

At autopsy in addition to macroscopic examination, the various organs were examined microscopically and culturally. The degree of infection was estimated in the various tissues of all the lambs by means of the technique already described and the colony counts are shown in Table XVI.

Microscopic Findings.

Microscopically the lesions in the various organs were typical of a pyogenic infection. The sections were stained by haematoxylin and eosin and also by Gram's stain. Various degenerative changes were noted and invariably a large number of polymorphonuclear leucocytes were present. A typical lesion involving the central nervous system of Lamb No. 444 is depicted in the accompanying microphotograph (see appendix). A severe meningitis was present in the cervical region of /

of the spinal cord of this lamb. The periosteum of the cervical vertebrae and the neighbouring muscles were also involved in the suppurative process.

Thus, typical pyaemic lesions developed in all the lambs which received 0.01 c.c. and 0.001 c.c. of staphylococcal suspension. Of the seven animals infected, five showed suppuration of the tissues adjacent to the central nervous system. Blood cultures could be obtained only from four of the seven lambs, twenty-four hours after infection. By means of colony counts of the viscera at autopsy it could be observed that the localisation of the infection was different in practically every animal examined. This fact correlates with our post-mortem findings in lambs brought to this Institute from tick-infested farms. One notable exception was the absence of liver lesions, visible to the naked eye, in experimentally infected animals. As stated previously, the liver is one of the commonest sites of abscess formation in the naturally occurring disease.

DISCUSSION AND CONCLUSION.

DISCUSSION.

The staphylococcal infection, frequently encountered in lambs on many tick-infested grazings throughout Britain, appears to be a specific enzootic type of disease. This disease is characterised by the development of suppurative foci in different parts of the body including the skin, musculature, tendon sheaths, joints, viscera and meninges. In addition to this pyaemic form of disease, an acute infection also occurs, the diagnosis of which can only be accomplished by bacteriological methods. Previous attempts at transmission of this staphylococcal infection by the subcutaneous inoculation of sheep by McFadyean (1894) and Taylor *et al.* (1941), failed to reproduce the typical disease. Despite the fact that the route of infection has not so far been determined, it appears probable that the common sheep tick, *Ixodes ricinus* L. plays some part in the transmission of the infection, either by providing suitable lesions for the entry of staphylococci from the skin of the lamb, or possibly, as a biological vector of the infective agent.

During the present work the majority of the organisms isolated from typical cases of pyaemia in lambs, were found to be closely related culturally and morphologically. From the nineteen strains subjected to examination, four were coagulase-negative varieties and /

and these strains lacked some of the characteristics associated with the other fifteen cultures. The notable features of these four strains were non-pathogenicity to the mouse and inability to produce toxins in any of the media employed. Their reactions on sugars were identical and it is of some interest that salicin was fermented by these four strains. The fermentation of this carbohydrate is not a usual feature of Staphylococcus aureus although it is reported that Staphylococcus citreus and Staphylococcus pharyngis both ferment this substance, Bergey (1939). Glycerol was also constantly fermented within four days by these four atypical varieties and each of these types produced a negative reaction to the Voges Proskauer test. The chromogenesis of these four strains differed from those of the other cultures examined. The colour produced on different varieties of media consisted of a pale yellow fawn shade which was quite distinct from the normal orange pigmentation associated with the other types of organisms. It may be noted that these pale strains were capable of giving rise to a slight orange colouration when grown upon potato slants for nine days, under suitable environmental conditions. It is probable that these non-coagulase types may have consisted of degenerate aureus forms.

The remaining fifteen strains produced very similar results in all the tests to which they were subjected. All were coagulase-positive and were proved to be pathogenic /

pathogenic to the mouse. They gave rise to bright orange pigmentation whengrown upon suitable media. Haemolysis of the α/β type occurred on sheep blood agar plates inoculated with thirteen of the fifteen strains. Two strains produced β toxin only, namely 691F and 71L. The biochemical reactions of the fifteen strains were almost identical and the cross-agglutination tests showed a considerable degree of serological relationship between the pathogenic types. In some cases it will be observed that organisms isolated from different organs in the same animal showed some degree of variation in their cultural and toxicogenic properties. These variations suggest the possibility that the organisms in those instances may have lost some of their original characteristics either in their particular location in the animal body, or during their subsequent cultivation on lifeless media prior to examination.

As a result of these cultural tests, a typical strain of the organism was selected and its pathogenic effect studied in greater detail in the guinea-pig and the young lamb. Following the intravenous injection of lambs, both the acute and pyaemic types of infection were produced experimentally. The chronic type of experimental infection closely resembled the naturally occurring pyaemic form of the disease encountered on tick-infested farms. By determining the degree of infection in the viscera of artificially infected lambs /

lambs, it was found that the organisms showed a tendency to localise in different organs in different animals. This againresembled the findings in natural cases of the disease, where great variation in the distribution of the lesions is encountered.

From the economic aspect it is essential that all possible prophylactic measures should be investigated with a view to diminishing the incidence of this infection. Methods are now available whereby louping-ill and the anaerobic infections of sheep can be controlled, but the blocking of these infections in the young lamb is of little value so long as the animal is still susceptible to infection with the staphylococcus. Apart from the general methods of tick-control and the use of derris powder applied to the newly born lamb, other means are required to diminish the incidence of this disease. The great advances at present being made in the utilisation of antibiotics such as penicillin in the treatment of pyogenic conditions, may yet provide a means whereby this serious disease can be suitably controlled.

CONCLUSION

As a result of these investigations it may be concluded that a specific enzootic disease caused by Staphylococcus aureus occurs in lambs on tick-infested farms; the disease may assume an acute or pyaemic form and both types of infection can be produced experimentally in mice, guinea-pigs and young lambs.

ACKNOWLEDGMENTS /

ACKNOWLEDGMENTS.

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APPENDIX.

TABLE I.

GELATIN LIQUEFACTION

5 DAY CULTURES AT 59°F.

Strain No.	Gelatin Plate Culture	Gelatin Stab Culture
19 L.	+++	+
71 L.	++	+
82 L.	++++	++++
84 L.	+++	++++
89 L.	+++	++
89 H.	+++	++++
90 J.	+	+
100 L.	+++	++++
106 P.	++	+
113 K.	+++	++
115 S.	+++	++
118 L.	+++	++
188 H.	+++	+
132 K.	+	+
134 L.	++	+
691 L.	+	+
691 K.	+	+
691 F.	+	+
691 T.	++	+

++++ signifies very good liquefaction
+++ " good liquefaction
++ " moderate liquefaction
+ " poor liquefaction

TABLE II.

CHROMOGENESIS

No. of Strain	Potato 48 hrs. at 37°C., then 7 days at room temperature	Loeffler's Serum 48 hrs. at 37°C., then overnight at room temperature	Plain Agar 48 hrs. at 37°C., then 5 days at room temperature	33 per cent. Milk Agar 48 hrs. at 37°C., then 2 days at room temperature
19 L.	orange	orange	orange	orange
71 L.	orange	orange	orange	orange
82 L.	very pale orange	pale yellow fawn	pale yellow fawn	pale yellow fawn
84 L.	orange	orange	orange	orange
89 L.	very pale orange	pale yellow fawn	pale yellow fawn	pale yellow fawn
89 H.	orange	orange	orange	orange
90 J.	very pale orange	pale yellow fawn	pale yellow fawn	pale yellow fawn
100 L.	orange	orange	orange	orange
106 P.	orange	orange	orange	orange
113 K.	orange	orange	orange	orange
115 S.	orange	orange	orange	orange
118 L.	orange	orange	orange	orange
118 H.	very pale orange	pale yellow fawn	pale yellow fawn	pale yellow fawn
132 K.	orange	orange	orange	orange
134 L.	orange	orange	orange	orange
691 L.	pale orange	pale orange	pale orange	pale orange
691 K.	pale orange	pale orange	pale orange	pale orange
691 F.	pale orange	pale orange	pale orange	pale orange
691 T.	pale orange	pale orange	pale orange	pale orange

TABLE III.

BIOCHEMICAL TESTS

Fermentation Reactions

Final readings at 7 days.* (No gas produced by any strains).
Incubated at 37°C.

Strain No.	Lactose	Maltose	Glycerol	Glucose	Sucrose	Mannitol	Salicin	Inulin	Raffinose	Dulcitol	Mannose	Sorbitol	Erythritol	Xylose	Galactose
19 L.	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+
71 L.	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+
82 L.	+	+	+	+	+	+	+	-	-	-	+	-	-	-	+
84 L.	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+
89 L.	+	+	+	+	+	+	+	-	-	-	+	-	-	-	+
89 H.	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+
90 J.	+	+	+	+	+	+	+	-	-	-	+	-	-	-	+
100 L.	-	+	-	+	+	+	-	-	-	-	+	-	-	-	-
106 P.	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+
113 K.	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+
115 S.	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+
118 L.	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+
118 H.	+	+	+	+	+	+	+	-	-	-	+	-	-	-	+
132 K.	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+
134 L.	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+
691 L.	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+
691 K.	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+
691 F.	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+
691 T.	+	+	-	+	+	+	-	-	-	-	+	-	-	-	+

* Except in the case of glycerol - in which readings were taken at 4 days.

\+ signifies acid production
\- " no reaction

TABLE IV

BIOCHEMICAL TESTS (CONTD.)

No. of Culture	Litmus Milk (incubated at 37°C. for 7 days)	Methyl Red	Voges Proskauer	Nitrate Reduction	NH_3 Production	Indole Production	H_2S Production	Methyl Blue Reduction
19 L	A. C. W.	+	+	+	+	-	-	+
71 L	A. C. W.	+	+	+	+	-	-	+
82 L	A. C. W.	+	-	+	+	-	-	+
84 L	A. C. W.	+	+	+	+	-	-	+
89 L	A. C. W.	+	-	+	+	-	-	+
89 H	A. C. W.	+	+	+	+	-	-	+
90 J	A. C. W.	+	-	+	+	-	-	+
100 L	Unchanged	+	+	+	+	-	-	+
106 P	A. C. W.	+	+	+	+	-	-	+
113 K	A. C. W.	+	+	+	+	-	-	+
115 S	A. C. W.	+	+	+	+	-	-	+
118 L	A.	+	+	+	+	-	-	+
118 H	A. C. W.	+	-	+	+	-	-	+
132 K	A. C. W.	+	+	+	+	-	-	+
134 L	A. C. W.	+	+	+	+	-	-	+
691 L	A. C. W.	+	+	+	+	-	-	+
691 K	A. C. W.	+	+	+	+	-	-	+
691 F	A. C. W.	+	+	+	+	-	-	+
691 T	A. C. W.	+	+	+	+	-	-	+

A signifies acid
C " clot
W " contraction of clot and expulsion of whey
+ " Positive Reaction
- " Negative Reaction.

TABLE V.

COAGULASE TESTS.

Incubated at 37°C. for the times specified below.

Strain No.	Human Plasma			Rabbit Plasma			Sheep Plasma			Horse Plasma		
	3 hrs.	5 hrs.	24 hrs.	3 hrs.	5 hrs.	24 hrs.	3 hrs.	5 hrs.	24 hrs.	3 hrs.	5 hrs.	24 hrs.
19 L	+	+	+	+	+	+	+	+	+	-	-	+
71 L	+	+	+	+	+	+	+	+	+	-	+	+
82 L	-	-	-	-	-	-	-	-	-	-	-	-
84 L	+	+	+	+	+	+	+	+	+	-	+	+
89 L	-	-	-	-	-	-	-	-	-	-	-	-
89 H	+	+	+	+	+	+	+	+	+	-	-	++
90 J	-	-	-	-	-	-	-	-	-	-	-	-
100 L	+	+	+	+	+	+	+	+	+	-	-	+
106 P	+	+	+	+	+	+	-	-	++	-	+	+
113 K	+	+	+	+	+	+	+	+	+	-	+	+
115 S	+	+	++	+	+	+	-	-	+	-	+	+
118 L	+	+	+	+	+	+	+	+	+	-	-	+
118 H	-	-	-	-	-	-	-	-	-	-	-	-
132 K	+	+	+	+	+	+	+	+	+	+	+	++
134 L	+	+	+	+	++	+	+	+	++	-	+	+
691 L	+	+	+	+	+	++	+	+	+	-	-	++
691 K	+	+	+	++	+	+	+	++	++	-	++	++
691 F	+	+	++	++	++	++	++	++	++	-	-	+
691 T	+	++	++	+	++	++	+	+	++	-	++	++

\+ signifies Firm Clot

\- " No change in the Plasma

TABLE VI

PATHOGENICITY TESTS IN MICE

No. of Strain	A. Intravenous Group — Doses in c.c.					B. Intraperitoneal Group — Doses in c.c.					C. Subcutaneous Group — Doses in c.c.		
	0.1	0.01	0.001	0.0001	0.00001	0.1	0.01	0.001	0.0001	0.00001	0.3	0.1	0.01
19 L	+) +)1 +)	+) +) 1 + 2	+ 3 L + 11 + 10	+ 10 S S	S S S	+) +) 1 +)	+L. 4 S S	S S S	S S S	S S S	+) +) 1	+ 1 + 2	S S
71 L	+ 2 + 6 + 8	+ 8 + 11 S	S S S	S S + 10	S S S	+ 8 S S	S S S	S S S	S S S	S S S	S S	S S	S S
84 L	+ 1 +) +)2	+) +) 3 + 5	+ 3 +) +) 6	+ 8 S S	S S S	+ 1 +L.4 S	S S S	S S S	S S S	S S S	+ 1 + 5	+ 7 S	S S
89 H	+) +)1 + 2	+ 2 + 4 S	S S S	S S S	S S S	+ 1 S S	+ 8 S S	S S S	S S S	S S S	+) +) 2	+ 6 S	S S
100 L	+) +)3 + 8	+ 7 + 8 S	+ 2 + 8 S	S S S	S S S	S S S	S S S	S S S	S S S	S S S	S S	S S	S S
106 P	+) +)1 +)	+ 1 + 2 +L.3	+ 1 + 3 + 4	+ 4 S S	S S S	+ 1 + 13 S	S S S	S S S	S S S	S S S	+) +) 1	+ 1 + 2	S S
113 K	+) +)2 + 5	+) +) 3 +)	S S S	S S S	S S S	+ 1 S S	S S S	S S S	S S S	S S S	+) +) 2	+ 5 + 10	S S
115 S	+ 1 +) +)2	+) +) 1 + 3	S S S	S S S	S S S	+) +) 2 S	S S S	S S S	S S S	S S S	+) +) 1	+ 3 + 6	S S
118 L	+ 1 + 2 + 4	+ 2 + 4 S	S S S	S S S	S S S	+ 1 + 2 S	S S S	S S S	S S S	S S S	+ 1 + 6	+) +) 2	S S
132 K	+ 4 + 9 +10	+ 3 S S	S S S	S S + 12	S S S	S S S	S S S	S S S	S S S	S S S	S S	S S	S S
134 L	+ 1 + 5 + 7	S S S	S S S	S S S	S S S	+) +) 1 S	S S S	S S S	S S S	S S S	+) +) 2	+ 2 S	S S
691 L	+) +)2 + 4	+ 2 + 5 + 8	+) +) 4 + 12	+ 8 S S	S S S	S S S	S S S	S S S	S S S	S S S	S S	S S	S S
691 K	+) +)1 +)	+) +) 2 + 3	+ 4 S S	S S S	S S S	+) +) 1 S	S S S	S S S	S S S	S S S	+) +) 1	+) +) 1	S S
691 T	+) +)1 + 2	+) +) 2 + L.4	+ 3 + 8 + 7	+ 8 S S	S S S	+) +) 1 +L.3	+ 3 S S	S S S	S S S	S S S	+) +) 1	+ 1 + 3	S S

By previous test 82 L, 89 L, 90 J and 118 H had been proved to be non-pathogenic.

L signifies, liver abscess
S " survival
+ " death

The numbers denote the time of death in days.

TABLE VII.

AGGLUTINATION AND CROSS-AGGLUTINATION TESTS

(Pathogenic Strains)

Strain No. agglutinogens	Sera prepared by inoculation of Rabbits with the specific antigens														
	19 L	71 L	84 L	89 W	100 L	106 P	113 K	115 S	118 L	132 K	134 L	691 L	691 K	691 F	691 T
19 L	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
71 L	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
84 L	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
89 H	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
100 L	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
106 P	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
113 K	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
115 S	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
118 L	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
132 K	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
134 L	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
691 L	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
691 K	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
691 F	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
691 T	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+

The agglutination titres obtained in these tests varried from 1/240 to 1/15,360. Against their own specific antigens, the sera usually gave high titres, e.g. Serum 691 F agglutinated strain 691 F in a dilution of 1/15,360. The average titre for all the strains was approximately 1/1,000.

\+ signifies Positive Reaction
\- " Negative Reaction.

TABLE VIII.

HAEMOLYSIS

5 per cent. Blood Agar plates. Surface colonies.

Strain No.	Sheep Blood 24 hrs. at 37°C.	Horse Blood 48 hrs. at 37°C.	Rabbit Blood 24 hrs. at 37°C.
19 L	P + C	-	C
71 L	P	-	-
82 L	-	-	-
84 L	P + C	-	C
89 L	-	-	-
89 H	P + C	C	C
90 J	-	-	-
100 L	P + C	-	C
106 P	P + C	-	C
113 K	P + C	-	C
115 S	P + C	-	C
118 L	P + C	-	C
118 H	-	-	-
132 K	P + C	C	C
134 L	P + C	-	C
691 L	P + C	-	C
691 K	P + C	-	C
691 F	P	-	-
691 T	P + C	C	C

P + C signifies a wide zone of partial haemolysis and a narrow clear zone at the periphery of the colony.

P " a wide zone of partial haemolysis extending from the edge of the colony.

\- " no haemolysis.

In sheep blood, after refrigeration, the partially haemolysed areas cleared considerably, especially those of strains 691 F and 71 L.

TABLE IX.

TOXIN PRODUCTION IN WALBUM'S MEDIUM.

9 day cultures grown in a 20 per cent. CO_2 atmosphere at 37°C.

Strain No.	M.H.D.	M.L.D. (Mouse)	M.R.D. (Guinea-pig)
19 L	0.00001 c.c.	0.0125 c.c.	0.00625 c.c.
71 L	0.002 c.c.	-	0.1 c.c. gave "flushing" only
82 L	-	-	-
84 L	0.00006 c.c.	0.05 c.c.	0.05 c.c.
89 L	-	-	-
89 H	0.00003 c.c.	0.3 c.c.	0.1 c.c.
90 J	-	-	-
100 L	0.00001 c.c.	0.025 c.c.	0.025 c.c.
106 P	0.00001 c.c.	0.0125 c.c.	0.0125 c.c.
113 K	0.00003 c.c.	0.1 c.c.	0.05 c.c.
115 S	0.00001 c.c.	0.0125 c.c.	0.00625 c.c.
118 L	0.0001 c.c.	0.5 c.c.	0.1 c.c.
118 H	-	-	-
132 K	0.00001 c.c.	0.025 c.c.	0.0125 c.c.
134 L	0.00006 c.c.	0.05 c.c.	0.025 c.c.
691 L	0.0003 c.c.	0.1 c.c.	0.05 c.c.
691 K	0.00001 c.c.	0.025 c.c.	0.0125 c.c.
691 F	0.0002 c.c.	-	0.1 c.c. gave "flushing" only
691 T	0.0003 c.c.	0.05 c.c.	0.025 c.c.

M.H.D. The smallest quantity of toxin which produced evidence of haemolysis of 1 per cent. sheep red blood corpuscles after a period of 1 hour at room temperature, 3 hours at 37°C. and finally, overnight refrigeration.

M.L.D. The smallest quantity of toxin which proved lethal to mice within 48 hours.

M.R.D. The smallest quantity of toxin which produced skin necrosis on the guinea-pig - readings taken at 48 hours.

\- signifies no reaction.

TABLE X.

THE DETERMINATION OF A SUITABLE INFECTIVE DOSE OF STRAIN 19L FOR THE GUINEA-PIG.

Inoculum		Result	Time of Death (in Days)
1 c.c.	Intracardially	+ +	2 1
0.5 c.c.	"	+	1
0.1 c.c.	"	+	2
0.05 c.c.	"	+ +	6 21
0.025 c.c.	"	+ +	5 5
0.0125 c.c.	"	+ + + + +	5 T.P. (lung lesions) 5 T.P. 2 T.P.(slight abscess formation) 5 T.P. 4 T.P.
0.0063 c.c.	"	+ + + L. L.	6 T.P. 8 T.P. 4 T.P. - -
0.0031 c.c.	"	L. L.	- -
0.0015 c.c.	"	L.	-

T.P. signifies Typical pyaemia.
L. " Animal lived.
+ " Animal died.

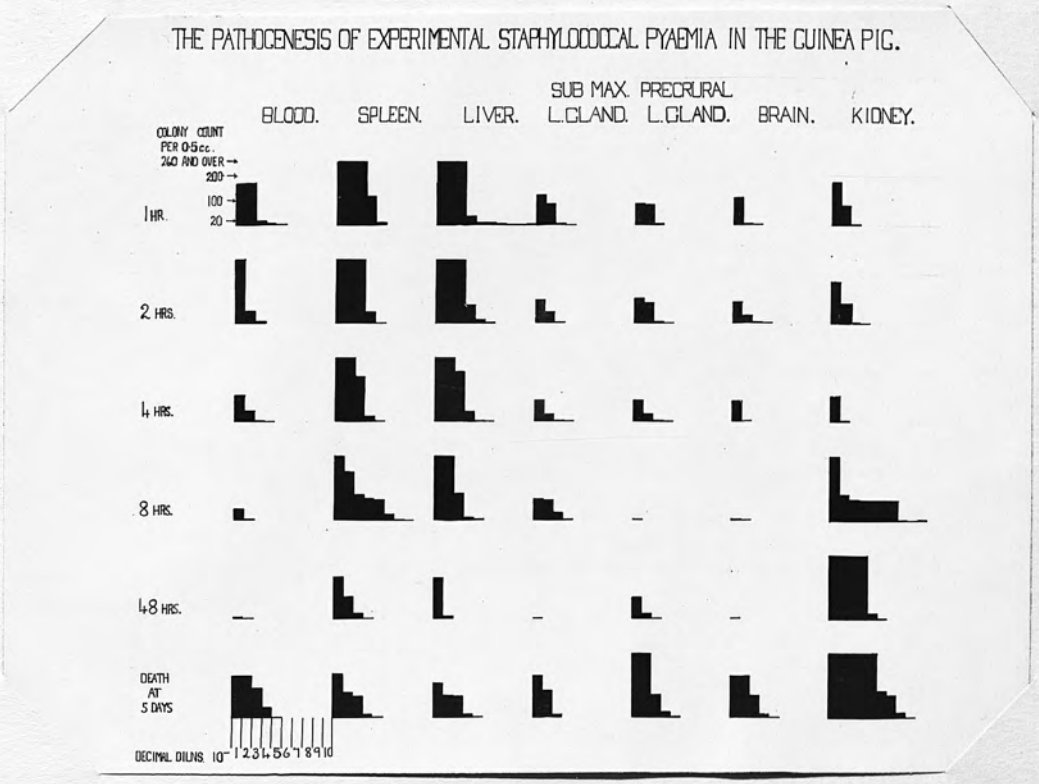
TABLE XI.

EXPERIMENTAL STAPHYLOCOCCAL INFECTION IN THE GUINEA-PIG.

No. of G. Pig	Inoculum	MACROSCOPIC LESIONS													Time of Death (days)
		Parietal Pleura	Pleural Fluid	Lungs	Peri-cardial fluid	Epi-cardium	Myo-cardium	Endo-cardium	Liver	Spleen	Kidneys	Sternal articu-lations	Costochondral articulations	Limb Joints	
1	1 c.c.	C	Ex.	C.H.	Ex.	C	-	C	C	C	SA.	-	-	-	2
2	1 c.c.	C	Ex.	C	Ex.	C	-	C	C	C	C	-	-	-	1
3	0.5 c.c.	C	Ex.	C	Ex.	C	-	C	C	C	C	-	-	-	1
4	0.1 c.c.	A	-	A	-	-	A	-	A	C	A	-	-	-	2
5	0.05 c.c.	A	-	A	-	-	A	-	C	C	A	-	-	-	6
6	0.05 c.c.	-	-	-	-	-	-	-	-	-	A	SA.	SA.	-	21
7	0.025 c.c.	-	-	-	-	-	A	-	C	C	A	SA.	SA.	-	5
8	0.025 c.c.	-	-	-	-	-	A	-	C	C	A	SA.	SA.	-	5
9	0.0125 c.c.	A	-	A	-	-	A	-	C	C	-	SA.	SA.	-	5
10	0.0125 c.c.	A	-	C	-	-	A	-	C	-	A	-	SA.	-	5
11	0.0125 c.c.	A	-	C	-	-	A	-	C	C	A	-	SA.	-	4
12	0.0125 c.c.	-	-	C	-	C	-	C	-	C	A	-	-	-	2
13	0.0125 c.c.	A	-	-	-	-	A	-	A	-	A	-	-	-	5
14	0.0063 c.c.	-	-	-	-	-	A	-	C	-	A	A	A	-	6
15	0.0063 c.c.	A	-	-	A	-	A	-	C	C	A	A	A	-	8
16	0.0063 c.c.	-	-	-	-	-	-	-	C	C	A	-	-	-	4
17	0.0063 c.c.	-	-	-	-	-	-	-	-	-	-	-	-	A-LE	S
18	0.0063 c.c.	-	-	-	-	-	-	-	-	-	-	-	A	-	S
19	0.0031 c.c.	-	-	-	-	-	-	-	-	-	-	-	-	-	S
20	0.0031 c.c.	-	-	-	-	-	-	-	-	-	-	-	-	-	S
21	0.0015 c.c.	-	-	-	-	-	-	-	-	-	-	-	-	-	S

A signifies marked abscess formation
S.A. " slight abscess formation
C. " Congestion
Ex. " Excess
H signifies Haemorrhage
L.E. " Left elbow joint
S " Survived
- " No lesion present.

CHART I.



The degree of infection is shown in the various organs of guinea-pigs inoculated intracardially with staphylococci.

TABLE XII.

ACUTE EXPERIMENTAL STAPHYLOCOCCAL INFECTION IN LAMBS

POST-MORTEM FINDINGS AND CULTURAL EXAMINATIONS.

ORGAN	LAMB No. 424	CULTURE	LAMB No. 425	CULTURE
Liver	Congested and darker than normal.	+	Congested and darker than normal	+
Spleen	Pin point haemorrhages in vicinity of periphery.	+	N.A.	
Kidneys & Bladder	Pin point white areas surrounded by zones of congestion. Urine in bladder, cloudy.	+ Urine also +	Pin point white areas surrounded by zones of congestion. Urine in bladder, cloudy.	+ Urine also +
Stomach & Intestines	N.A.		N.A.	
Peritoneal Cavity	Small petechiae along retro-peritoneal region.		Slight excess of blood-stained peritoneal fluid.	
Lungs	Small areas of congestion and haemorrhages in all lobes of both lungs	+	Petechial haemorrhages in all lobes of both lungs.	+
Heart	Slight excess pericardial fluid. Haemorrhages in epi- myo- and endocardium.	H.B. + P.F. +	Slight excess of pericardial fluid.	H.B. + P.F. +
Pleural Cavity	Petechiae in dorsal aspect of thoracic cavity in vicinity of aorta.		Slight excess of blood-stained pleural fluid.	
Central Nervous System	N.A.	C.S.F. — (L.P.)	N.A.	C.S.F. — (L.P.)
Skin, Joints & Musculature	Left stifle joint contained blood-stained synovia. Other joints apparently normal.	All joints examined +	Left elbow joint contained blood-stained synovia. All other joints apparently normal.	All joints examined +

N.A. signifies No abnormality.
\+ " Culture of coagulase + staphylococci with α / β haemolysis.
\- " No staphylococci isolated.
H.B. " Heart blood.
P.F. " Pericardial fluid.
C.S.F.(L.P.) " Cerebral spinal fluid withdrawn by lumbar puncture.

'All joints examined' denotes shoulder, elbow, knee, stifle and hock joints.

CHART II.

THE TEMPERATURE REACTIONS OF LAMBS AFFECTED WITH EXPERIMENTAL PYAEMIA.

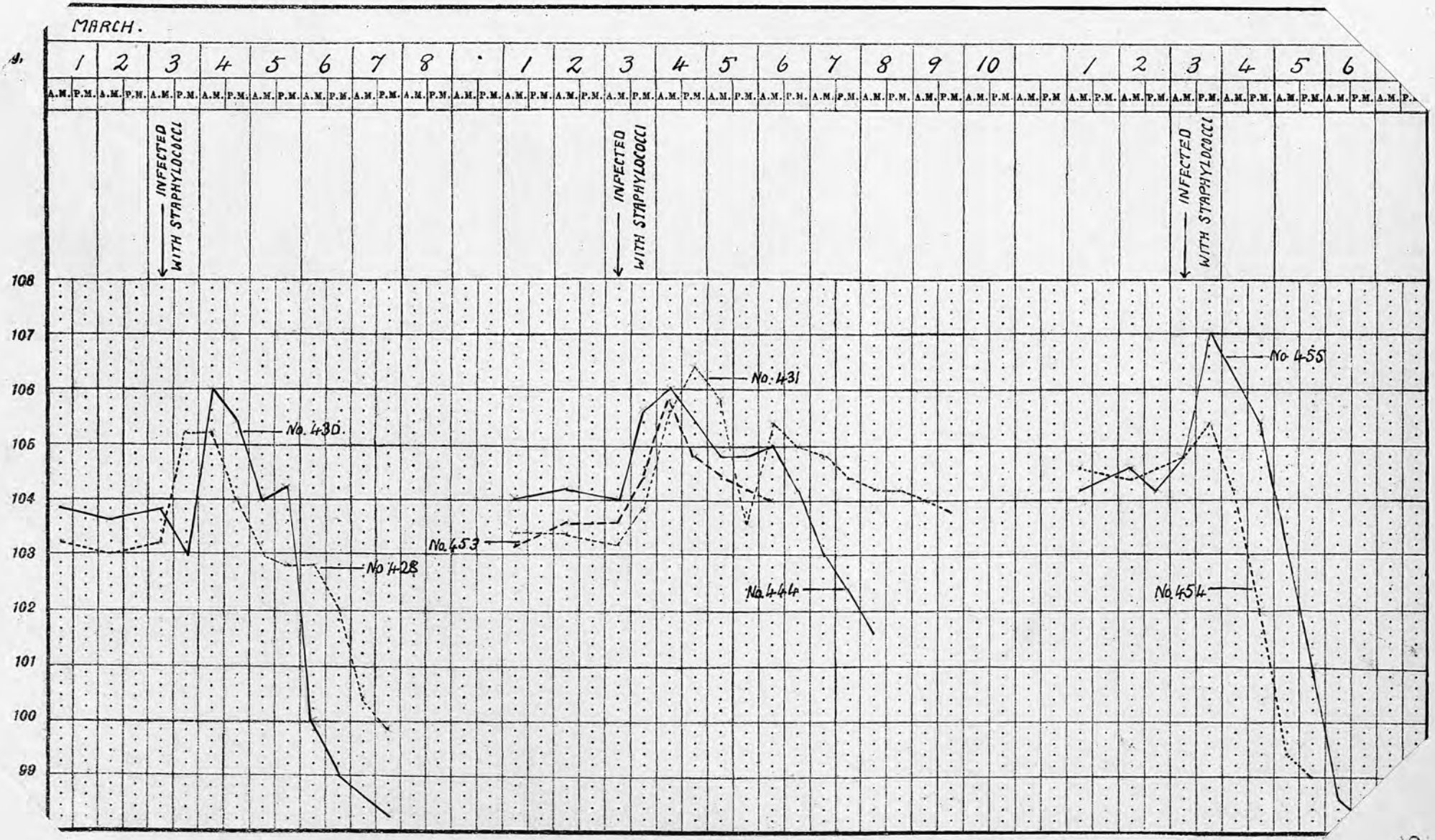


TABLE XIII.

BLOOD COLONY COUNTS DURING LIFE FROM LAMBS AFFECTED WITH EXPERIMENTAL PYAEMIA.

Number of Lamb	Time of Removal of Blood Samples from Jugular Vein after Infection							
	4 hrs.	24 hrs.	48 hrs.	72 hrs.	4 days	5 days	6 days	7 days
428	3	5	-	-	-	Died		
430	-	-	-	-	-	Died		
431	-	-	-	-	-	-	-	Died
444	-	3	-	-	-	Died		
453	-	123	-	Died				
454	-	-	-	Died				
455	-	200	-	-	Died			

The numbers signify the number of colonies on 5 per cent. sheep blood agar plates spread with 0.5 c.c. of 10^{-1} dilution of blood in normal saline.

— signifies No staphylococci isolated.

TABLE XIV.

CLINICAL SIGNS IN EXPERIMENTAL STAPHYLOCOCCAL PYAEMIA IN LAMBS.

No.of Lamb.	Days after Infection.						
	1	2	3	4	5	6	7
428	'Knuckling'at both knee joints. Mucous membranes congested. Jerky respirations. Bright appearance. Feeding well.	'Trembling', lame in forelegs. Fluctuation(right elbow)willing to suck if held to the ewe.	Dull, comatose,profuse salivation. Diarrhoea,trembling.	As for previous day but much more comatose.	Died during the night.		
430	Lame - left foreleg. Mucous membranes congested.	Marked lameness left foreleg. Mucous membranes congested. Left knee swollen and fluctuating.	Much weaker. Disinclined to stand, still feeding.	Very weak. Unable to stand. Profuse salivation,cold extremities.	Died during the night		
431	Slight congestion of mucous membranes. Increased respirations.	Dull,lame - left hind leg. Slight swelling of right knee.	Slight swelling of right knee. No lameness. Feeding well.	Much brighter in appearance. Following ewe. Feeding well. No lameness.	Apparently normal. Feeding Well.	The lamb appeared to have recovered.	Sudden death of the lamb.
444	Increased respirations. Slight congestion of mucous membranes.	Very weak. Unable to stand. Slight frothing at mouth.	No lameness, able to rise easily, salivating.	Comatose. Unable to rise. Much salivation,cold extremities.	Died early in the forenoon.		
453	Increased respirations when forced to move.	Dull,disinclined to move, mucous membranes congested. Appeared stiff. Much salivation.	Comatose. Died in the afternoon.				
454	Slight incoordination of gait. Congested mucous membranes.	Marked incoordination of gait. Slight salivation.	Unable to stand. Comatose. Died durthe forenoon.				
455	Increased respiratory rate. Slight congestion of mucous membranes.	Irregular respirations. Stiff right hind leg. The limb was dragged when the animal moved.	Right hindleg extended rigidly. Head twisted to left side. Very like 'louping-ill.'	Comatose. Died approximately at mid-day.			

TABLE XV.

EXPERIMENTAL STAPHYLOCOCCAL PYAEMIA IN LAMBS
POST-MORTEM FINDINGS AND CULTURAL EXAMINATION

ORGAN	No.428	No.430	No.431	No.444	No.453	No.454	No.455
Liver	Congested. C+	Slightly enlarged & congested. C+	Soft, friable, some patchy congestion.	Congested and paler areas in central lobe. C+	Congested. C+	Slightly enlarged and congested. C+	Congested
Spleen	NA. C+	Congested. C+	Enlarged & Congested.	NA. C+	NA. C+	NA. C+	NA. C+
Kidneys & Bladder	Small abscesses in substance of both kidneys. C+	Markedly congested. C+	Small abscesses in substance of both kidneys. C+	Abscesses in both kidneys. C+	Marked congestion. C+	Marked congestion. C+	Abscesses in both kidneys C+
Stomach & Intestine	Peyer's patches congested.	NA.	NA.	NA.	NA.	NA.	NA.
Peritoneal cavity	Mesenteric glands enlarged. Excess peritoneal fluid.	NA.	NA.	NA.	NA.	NA.	NA.
Lungs	Small abscesses in all lobes. C+	Small abscesses in all lobes. C+	Small zones of red hepatisation.	Small abscesses in all lobes. C+	Small abscesses in all lobes. C+	Small abscesses in all lobes. C+	Small abscesses i all lobes. C+
Heart	Pericarditis Epicarditis Myocardial abscess. (H.B.) C+	Pericarditis Epicarditis Myocardial abscess (H.B.) C+	Pericarditis. Myocardial abscess ruptured into ventricle. (H.B.) C+	Pericarditis Endocarditis Myocardial abscess. (H.B.) C+	Excess pericardial fluid Myocardial abscess. (H.B) C+	Pericarditis Myocardial abscess. (H.B) C+	Pericarditis Myocardial abscess. (H.B. C+
Pleural Cavity	Pleurisy	Pleurisy with adhesions.	Pleurisy	Pleurisy	Pleurisy with adhesions.	Pleurisy	Pleurisy
Central Nervous System & Adjacent Tissue.	NA.	Abscess in periosteum of temporal bone.	Abscesses in thoracic, cervical and lumbar regions, adherent to meninges.	Abscess in frontal bone. Abscess in thoracic region adherent to meninges.	Abscesses in temporal & occipital bones, cervical mengitis.	NA.	Purulent thoracic meningitis. Abscess in lumbar vertebrae.
Skin & Musculature.	Abscess in superficial muscles, especially mandibular region, groin & axilla.	Numerous abscesses in skin & muscles, especially axillary region.	A few abscesses in gracilis & sartorius muscles.	NA.	NA.	NA.	NA.
Joints	Purulent synovitis in right stifle. Excess synovia in right & left shoulder joints. All C+.	Suppurative arthritis in right shoulder joint, left knee joint & tendon sheaths. All C+.	NA. in limb joints. Costochondral abscesses gave C+.	NA. in limb joints. Costochondral abscesses gave C+.	NA. in limb joints. Costochondral abscesses gave C+.	NA.	NA.

C+ signifies Culture of staphylococcus, coagulase-positive and producing α/β haemolysis.

NA. " No abnormality.

HB. " Heart Blood.

TABLE XVI.

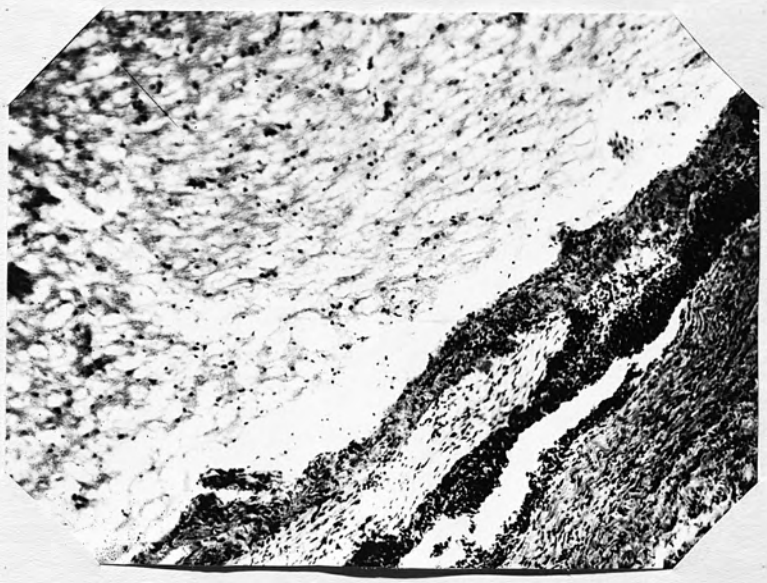
THE DEGREE OF INFECTION IN THE VARIOUS ORGANS OF LAMBS AFFECTED WITH PYAEMIA.

No. of Lamb.	ORGAN	DILUTIONS									
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
428	Blood (H)	++	++	++	156	12	-	-	-	-	-
	Lung	116	14	3	-	-	-	-	-	-	-
	Liver	43	5	-	-	-	-	-	-	-	-
	Spleen	++	++	163	13	1	-	-	-	-	-
	Kidney	++	++	++	++	184	27	2	-	-	-
	Submaxillary L.Gland	++	++	++	++	157	3	-	-	-	-
	Precrural L.Gland	++	++	++	84	6	-	-	-	-	-
430	Blood (H)	2	-	-	-	-	-	-	-	-	-
	Lung	10	3	1	-	-	-	-	-	-	-
	Liver	2	-	-	-	-	-	-	-	-	-
	Spleen	++	++	36	4	-	-	-	-	-	-
	Kidney	++	++	++	++	127	11	-	-	-	-
	Submaxillary L.Gland	22	3	-	-	-	-	-	-	-	-
	Precrural L.Gland	++	++	++	82	5	-	-	-	-	-
431	Blood (H)	++	++	217	36	6	-	-	-	-	-
	Lung	-	-	-	-	-	-	-	-	-	-
	Liver	-	-	-	-	-	-	-	-	-	-
	Spleen	-	-	-	-	-	-	-	-	-	-
	Kidney	++	++	57	4	1	-	-	-	-	-
	Submaxillary L.Gland	-	-	-	-	-	-	-	-	-	-
	Precrural L.Gland	-	-	-	-	-	-	-	-	-	-
444	Blood (H)	143	15	-	-	-	-	-	-	-	-
	Lung	10	4	-	-	-	-	-	-	-	-
	Liver	14	-	-	-	-	-	-	-	-	-
	Spleen	29	4	-	-	-	-	-	-	-	-
	Kidney	++	++	++	++	6	-	-	-	-	-
	Submaxillary L.Gland	-	-	-	-	-	-	-	-	-	-
	Precrural L.Gland	17	1	-	-	-	-	-	-	-	-
453	Blood (H)	++	++	90	10	-	-	-	-	-	-
	Lung	++	++	++	++	60	5	-	-	-	-
	Liver	30	6	-	-	-	-	-	-	-	-
	Spleen	++	++	++	++	++	38	4	-	-	-
	Kidney	++	++	92	12	-	-	-	-	-	-
	Submaxillary L.Gland	149	22	3	-	-	-	-	-	-	-
	Precrural L.Gland	-	-	-	-	-	-	-	-	-	-
454	Blood (H)	++	++	220	32	4	-	-	-	-	-
	Lung	++	++	++	100	10	-	-	-	-	-
	Liver	++	++	++	26	1	-	-	-	-	-
	Spleen	++	++	++	++	75	16	1	-	-	-
	Kidney	++	++	++	30	10	1	-	-	-	-
	Submaxillary L.Gland	-	-	-	-	-	-	-	-	-	-
	Precrural L.Gland	32	3	-	-	-	-	-	-	-	-
455	Blood (H)	++	++	++	86	12	-	-	-	-	-
	Lung	3	1	-	-	-	-	-	-	-	-
	Liver	-	-	-	-	-	-	-	-	-	-
	Spleen	10	3	-	-	-	-	-	-	-	-
	Kidney	16	3	-	-	-	-	-	-	-	-
	Submaxillary L.Gland	4	-	-	-	-	-	-	-	-	-
	Precrural L.Gland	2	-	-	-	-	-	-	-	-	-

Inoculum for each plate = 0.5 c.c.
The figures signify the number of colonies counted.
++ signifies above 260 colonies.
- " no colonies present.

PYAEMIC SPINAL MENINGITIS

Lamb No.444.



(haematoxylin and eosin)

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